SYMPOSIUM ABSTRACTS

S1 ICMSF Symposium on International Developments in Food Safety

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Food safety management is a multistakeholder responsibility, involving government providing guidance and regulation on sound approaches to food safety management and targets for control of hazards in the food chain next to food industry professions that are responsible day-to-day to assure the safety of food products offered to consumers. Modern food safety management considers consumer risk in designing effective food safety management and new concepts have been introduced in establishing operational control measures for and in industry. Testing is an integral part of food safety management systems, with an emphasis on testing to verify the food production system is running as designed. This symposium highlights some of the new develoments in modern food safety management and points out useful testing approaches and associated sampling schemes. Examples are given for several important commodities, including meat and poulty, produce and seafood.

S2 Sterilant Gas Decontamination of Food and Environments and Emerging Technology

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Sterilant gas technologies show promise for disinfection of many types of foods as well as facilities and equipment including those that process foods. As gases, they can penetrate into crevices and niches in foods and in facilities where entrapped microbes could be missed by conventional techniques of cleaning and sanitization.

The principles of sanitary facility and equipment design have been known for many decades. Yet development of microbial growth niches in equipment and facilities has been, and continues to be, a frequent source of contamination to process foods and other items. Many of these niche sites are difficult (and in some cases impossible) to disassemble for effective cleaning and sanitization and they entrap microbes which may grow to high numbers resulting in contamination of items in these environments. Traditional means of cleaning and sanitation do not effectively remediate these areas. Sterilant gas treatments hold some promise to penetrate and disinfect hard to reach areas in facilities and foods and thereby save future generations the pain of contaminated food, equipment, facilities, etc. This mini-symposium will provide the latest on pros, cons, legalities, and the efficacy of emerging sterilant gas technologies to decontaminate foods and facilities.

S3 Harnessing Irradiation for the Marketplace Today

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Scientists, regulators and lawmakers have been working for years to harness irradiation to combat foodborne illnesses. In August of 2008 FDA approved the use of low-dose irradiation for iceberg lettuce and spinach. In addition, USDA FSIS expressed interest in approving irradiation as a processing aid on carcasses in beef slaughter establishments to better control pathogens. These advancements are very encouraging for the consumer, as the use of these new technologies can substantially reduce the food safety risks of meat and fresh produce. Decades of scientific data have shown that irradiation can be safely applied as an effective tool in helping to control foodborne pathogens. It is but one additional tool for industry to apply as an integral part of a comprehensive program to enhance food safety. Irradiation, which involves exposing food briefly to radiant energy (such as gamma rays, high-energy electrons, or X-rays), can reduce or eliminate microorganisms that cause foodborne disease without significantly changing the quality of the food. This session will focus on the regulatory, operational and consumer educational aspects of applying irradiation today to enhance consumer confidence and reduce foodborne illnesses.

S4 Epidemiological Trends of Noroviruses

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Recent outbreaks of GII.4 norovirus strains implicate incidences of high death rates in the immunocompromised, the elderly, and perhaps even healthy individuals. The changing genetic drift of noroviruses implicates a crucial role in their transmission along with an increase in the severity and mortality of the disease burden. Efficacious control

strategies implemented by the Vessel Sanitation Program (CDC, USA) should decrease cruise ship related outbreaks. Novel food processing trends and improved detection methods implicate outbreaks of noroviruses in RTE foods as observed in the Grand Canyon rafting outbreak. The recent institutional outbreak resulting in >400 cases in a Texas prison also show persistence and transmission trends of noroviruses. Improved detection, new processing technologies, and novel intervention controls can shed light towards the persistence, transmission, and effective control of norovirus related outbreaks. This symposium focusing on recent norovirus outbreaks and intervention strategies will help the public health sector, the food industry, and academia remain current in issues related to noroviruses.

S5 Pathogen and Spoilage Persistence in the Processing Environment and Food Products: Where, Why and How Do We Know

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While the phenomenon and importance of "persistence" has been recognized since "Typhoid Mary," the use of molecular subtyping methods for characterizing both foodborne pathogens and spoilage microorganisms has increased our understanding and appreciation of microbial persistence throughout the food chain. This symposium will address microbial persistence from primary production to processing, including the importance of persistence in foodborne disease outbreaks. Attendees will gain an understanding of microbial persistence, including the importance of controlling persistence and understanding persistence throughout the food chain.

S6 Zapped! Optimizing the Consumer Experience of Microwave Cooking through Labeling, Infrared Thermography, and Validation

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STEVE VLOCK, ConAgra Foods, Omaha, NE, USA
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Consumers do not know the wattage of their microwave ovens and this lack of knowledge may contribute to improperly cooked not-ready-to-eat foods. A small working group comprised of frozen food manufacturers, retailers, microwave oven manufacturers and the American Frozen Food Institute have been working together in the last year to give consumers more tools to prepare products properly. The working group has developed a wattage labeling protocol for microwave ovens, identified a symbol for microwave wattage information, and developed usage of that symbol on microwave food cooking directions. This comprehensive program has recently rolled out at Target. Details and background of this initiative will be shared. We look forward to the entire industry continuing the partnership for microwave food safety.

A common problem with microwave-heated foods is poor uniformity of heating leading to the formation of hot and cold spots. Thermal imaging is an invaluable tool in determining the location and temperature of such problem areas. This presentation discusses the advantages and limitations of thermal imaging used to aid the development of microwave products and microwave instructions and to help understand the variability in heating between different microwave ovens.

Validating microwave oven cooking directions is essential not only to food safety but also to product performance and quality. Creating an in-house microwave validation lab can be an ideal way to test current products as well as R&D work for future projects. There are numerous factors to be considered when creating a validation lab such as: number of microwaves, brand, size, labeled wattage, actual wattage, electrical wiring, wall voltage, equipment, calibration, and product storage. Certain factors may be more critical to certain applications but all should be considered. An overview of each one of these will be discussed during the presentation.

The American Frozen Food Institute (AFFI) has a long standing relationship, going back more than 20 years, with the microwave industry and its members to ensure the safe, effective use of this technology. During the last 10 years, frozen food processors have dramatically enhanced the quality and innovative characteristics of foods suitable for microwave cooking. At the same time, advancements in packaging and new microwave oven technologies have given consumers an abundance of options when it comes to foods able to be prepared in the microwave.

In 2007, AFFI introduced the consumer guide, "Cooking with Microwave Ovens – Nutrition and Food Safety Considerations," which we offered to our members for their use and dissemination. In 2008, AFFI completed a package labeling guide for microwaveable foods. This guide, which was distributed widely in the food industry, will assist frozen food and other food processors in the development of labels and microwave cooking instructions that are more easily understood by consumers.

The guide is also intended to foster widespread industry use of more consistent language in microwave cooking instructions.

More recently, AFFI has chaired the Microwave Working Group, a coalition of microwave manufacturers, food processors and microwave retailers whose goal is to help foster programs which will enhance the clarity and consistency of microwave cooking. Several months ago we launched a Web site, www.microwaveovenfacts.com to help educate consumers, and are currently chairing a task force sponsored by the Partnership for Food Safety Education, in conjunction with the U.S. Department of Agriculture's, Food Safety Inspection Service (USDA/FSIS) and Food and Drug Administration (FDA) to develop consumer messages and materials regarding safe microwave cooking. This presentation will discuss some of those activities.

S7 *Listeria monocytogenes* Controls from Local to Global – Are They Working?

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While *Listeria monocytogenes* controls have been implemented to reduced or eliminate the risk of this foodborne pathogen in the food supply, are these controls sufficient to protect the consumer? The range of *Listeria* control strategies can vary depending on the type of food product, size of the processing operation, and regional regulations. This symposium summarizes the various *L. monocytogenes* prevention practices that are used in small farmstead operations, North American controls established through regulations and/or guidance documents, and internationally recognized standards in Codex Alimentarius. Adequacy of established *Listeria* controls are discussed as well as highlights of new or proposed control strategies.

S8 The Effect of Climate Change on Food Availability and Safety

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Climate change is inevitable with implications for all types of food: fish, shellfish, animal products and crops. It will affect different parts of the world in different ways, but generally the developing countries in tropical and subtropical regions are likely to fare the worst. Some effects are noticeable today, particularly in areas with increasing drought and shrinking ice cover in the seas. However, we expect this process to accelerate in the near future, even if carbon emissions slow down of the next few years. This will mean more severe weather patterns and more food shortages. The effects of climate change on food security are being explored but those on food safety are poorly understood. Starting to explore these questions are speakers from the Food and Agriculture Organization, government and academia. Symposium speakers will present an overview of the effects of climate change on food security and food safety. The types of questions to be addressed will include the following. Will elevated temperatures result in harmful algal blooms and increase the number of foodborne outbreaks associated with vibrios? Will fungal diseases destroy essential grain crops and increase the amount of mycotoxin production? How will our food animals adapt to increases in temperature and possibly a lack of food and water? Will new breeds be developed to meet these demands or will production move to new geographical areas? Will GMOs in crops, fish and animals become increasingly important as our response to climate change? In view of the recent increase in extreme weather events will we have a safe and adequate food supply? The FAO has written a recent monograph on the Implications of Climate Change for Food Safety which will be drawn upon by some of the speakers.

S9 Tracking and Tracing Technologies – Do You Know Where Your Steak and Tomatoes Come From?

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The desire for cheap, available and affordable food for all people has led to a focus on traceability of products by industry from ingredients to the consumers' homes. Investigation of recent contamination events and outbreaks of illness has been thwarted by lack of proper traceability, e.g., melamine in pet food and milk ingredients, dioxin in animal feed, *Salmonella* in cantaloupes, tomatoes and peppers. Both deliberate adulteration and worker error have contributed to such problems, and means of guaranteeing delivery of safe product down the food chain from company to company is critical. In this symposium we will be exploring the use of electronic tracking systems operating in different countries but with an aim of eventual acceptance of universal standards. Tags with barcodes have been used by the food industry for some time, but RFID technology is making advances to make this easier at the pallet level. A future goal is go to the retail portion level which will help with more rapid recalls. Animals with ear tags can be traced from slaughterhouse back to the farm, but DNA identification methodology now can go further linking fabricated meat to individual animals after slaughter. Produce traceability represents unique challenges because of the perishability of the products, they way the industry mixes items from different sources, and their generally small item size, but the industry is exploring innovative ideas to overcome such challenges. The drug and other industries have been using tracking systems for their products for several decades, especially for adulteration and fraud issues, and we can learn from them how to adapt their methods to food products.

S10 International Food Protection Issues: Overview and Global Commodity Trade

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LARRY KEENER, International Product Safety Consultants, Seattle, WA, USA
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ROBERT L. BUCHANAN, University of Maryland, College Park, MD, USA

The proposed symposium is a new topic for IAFP that addresses scientific, policy, and communication issues vital to improving the global food supply. The symposium will provide a snapshot of initiatives underway to harmonize global food safety practices and regulations and, through means of an interactive case study, highlight the challenges associated with establishing and meeting global food and ingredient safety standards.

S11 Foodborne Disease Outbreak Update: *Campylobacter* in Fresh Peas; *Salmonella* Schwarzengrund in Pet Food; *Salmonella* Saintpaul in Tomatoes/Peppers

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COLLETTE FITZGERALD, CDC, Atlanta, GA, USA
IAN WILLIAMS, CDC, Atlanta, GA, USA
SHERRI A. MCGARRY, FDA, College Park, MD, USA

This symposium will discuss three different outbreaks. The first outbreak involves contamination of fresh peas with *Campylobacter* in Alaska and the investigation to identify the source of the contamination as Sand Hill Cranes. CDC will also report on the intensive laboratory investigation they undertook to confirm this link. The second outbreak describes the link between pet food and human cases of *Salmonella* Schwarzengrund. Two presentations will first describe the epidemiological investigation and then FDA's investigation and response to the outbreak. The third pair of speakers will discuss the 2008 *Salmonella* Saintpaul outbreak linked to the consumption of tomatoes and peppers. What do we know one year later and what are the answers to some of the unanswered questions?

S12 Attribution of Foodborne Illness/Disease

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TRACY AYERS, CDC, Atlanta, GA, USA
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Different approaches are used to prioritize efforts to control foodborne diseases. Generally, the efforts are aimed at the pathogens most commonly associated with human disease, those associated with the largest numbers of outbreaks, or those causing the greatest burden to public health. A more recent approach is directed toward the food sources/vehicles most commonly associated with human disease. Attribution analysis is the science that estimates the relative contribution of different commodities to the burden of foodborne disease. This symposium will begin with the newest estimates of the burden of foodborne illness in the United States and continue with an introduction to the concept of attribution analysis with examples from the United States and abroad.

S13 Best Practices for Cleaning and Validation

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Hygiene and sanitation of manufacturing equipment used in the production of foods are essential for ensuring the resulting products are safe and of high quality. While the concept of cleaning is easy to grasp, the practical meaning of "clean" and how to balance cleaning and cleaning verification with time and budget constraints is a significant issue. There are numerous options in cleaning systems and chemicals – and nearly as many for cleaning verification systems. With such an array of options available, it is important to focus on the core objectives of a cleaning system and the best practices for meeting those objectives. This symposium will look at the best practices for cleaning and cleaning verification/validation. After an overview of the practical importance of cleaning, the methods of wet cleaning will be discussed in more detail. Best practices for the implementation of ATP hygiene monitoring systems will be explained with practical examples of how to maximize the value obtained from each ATP testing dollar. The focus will then shift to dry cleaning methods and the validation of dry cleaning methods. Finally, the various methods for environmental allergen testing will be discussed.

S14 Enhancing Oyster Safety through *Vibrio* Control Plans

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MARYANNE GUICHARD, Division of Environmental Health, Dept. of Health, Tumwater, WA, USA
ANGELO DEPAOLA, FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, USA
MIKE VOISIN, Motivatit Seafoods, Houma, LA, USA

The Interstate Shellfish Sanitation Conference (ISSC) adopted a *Vibrio parahaemolyticus* control plan that was implemented in June 2008. In order to reduce *V. parahaemolyticus* levels the time from harvest to first refrigeration was adjusted according to monthly water temperatures. FDA developed a risk calculator based on its *V. parahaemolyticus* risk analysis to estimate the impact of time to refrigeration on *V. parahaemolyticus* levels at risk to determine control measures to be enforced. A similar approach is being used for a *Vibrio vulnificus* plan based on a FAO/WHO *V. vulnificus* risk analysis that will be implemented in 2010. This symposium will demonstrate the use of these risk calculators. Additionally industry representatives will provide their prospective on the impact of this plan on harvesting, processing and retail. The view of state regulations on implementation of this plan will also be presented. Finally the impact of the *V. parahaemolyticus* control plan and illness reporting will be evaluated by epidemiologists.

S15 Less Recognized and Underappreciated Foodborne Pathogens – No Crystal Ball for the Next Big Bug

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MARK D. SOBSEY, Dept. of Environmental Sciences and Engineering, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA
MICHAEL T. COLLINS, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA
SURESH D. PILLAI, Texas A&M University, College Station, TX, USA

Many pathogens of significance to the food industry (e.g., *Listeria monocytogenes*, *E. coli* O157:H7, *Enterobacter sakazakii* in infant formula, *Salmonella* Enteritidis in Grade A eggs) were at one time either unheard of or under-appreciated. Surviving today's global economy demands a forward-thinking approach to food safety, which includes not only diligence with respect to established pathogens, but vigilant awareness to potential, obscure and less recognized microorganisms. Although there is no crystal ball, those associated with the food industry should maintain a sense of alertness to rumblings of fringe pathogens that might become tomorrow's scourge. This symposium addresses not only what are commonly called emerging pathogens, but digs one layer deeper to elucidate many microorganisms infrequently discussed. These pathogens are often overlooked yet possess the propensity to reshape the food industry, given the proper set of conditions. This series of lectures will interest those from industry, academia and government by sounding the alarm without sounding alarmist to ready ourselves for the next big bug.

S16 Facing a Persistent Challenge: *Salmonella* Control in Low-moisture Foods

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LES SMOOT, Nestle, Dublin, OH, USA
JEFF BANKS, Cadbury, London, United Kingdom
IAN WILLIAMS, CDC, Atlanta, GA, USA
JENNY SCOTT, Grocery Manufacturers Association, Washington, D.C., USA
THEODORA MORILLE-HINDS, Kraft Foods, Tarrytown, NY, USA

A second major *Salmonella* outbreak in the US linked to peanut butter highlights the difficulty and importance of *Salmonella* control in low-moisture products. Several significant outbreaks of salmonellosis have been linked to foods produced in dry manufacturing environments. Human illnesses have been attributed to the handling of contaminated dry pet foods and pet chews, as well as the consumption of a wide variety of contaminated low-moisture products including peanut butter, chocolate, breakfast cereals, snacks, nuts, infant formula and powdered milk. The control of *Salmonella* in these products and their manufacturing environments is difficult and highly specialized, with few practical references available. This symposium will draw upon a guidance document summarizing extensive industry experience and available scientific data and information. It will serve as a medium for sharing industry practices for *Salmonella* control in low-moisture products that will enable industry to more effectively reduce the risk of *Salmonella* contamination in this major food category. Global adoption of effective control measures can greatly benefit overall public health protection.

S17 Food Safety Challenges Impacting Global Food Trade

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ALEJANDRO CASTILLO, Texas A&M University, College Station, TX, USA
CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, Washington, D.C., USA
SUELY M. NAKASHIMA, Sadia, São Paulo, Brazil

This Symposium will provide a forum where food importing countries and food exporting countries will share their experiences in regulating and ensuring compliance with food safety regulations. For the exporter this includes ensuring compliance with the domestic regulations and requirements of importing countries, and for the importing country there is the challenge of how to assess food safety management systems in the exporting country when the product reaches their border. The Symposium will include perspectives from both importing and exporting countries on how food safety laws function and the importance of harmonization in improving efficiency and allowing seamless trade in food commodities. Cultural aspects of food safety and how food safety information is communicated between countries and regions will also be discussed.

S18 Looking for Thresholds: A Multi-disciplinary Key Events Approach

ROBERT L. BUCHANAN, University of Maryland, College Park, MD, USA
MARY ALICE SMITH, University of Georgia, Griffin, GA, USA
RICHARD C. WHITING, Exponent, Bowie, MD, USA
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Consumers are exposed to a range of substances in food – i.e., inherent constituents (micronutrients, proteins, etc.) and also contaminants introduced during food production or processing (preservatives, microbial pathogens, pesticide residues, etc.). Both types of substances can pose a potential health risk if consumed by susceptible individuals or at high enough levels. Traditionally, the various sub-disciplines in the field of food safety have used discipline-specific approaches to set regulatory standards and protect consumers. Each sub-discipline has developed its own approach to determining whether safe levels of exposure or "thresholds" exist – and if so, what those safe levels are. But with recent scientific advances, these disciplines are moving towards a more mechanistic understanding of the biological events that occur between initial exposure to a substance and the ultimate effect of concern. These advances provide a common platform

for revisiting the various discipline-specific approaches to identifying thresholds and setting "safe" exposure levels. After convening a cross-disciplinary working group, with experts in pathogenic microbiology, allergenicity, nutrition, toxicology and risk assessment, the ILSI Global Threshold Project adopted a novel approach for evaluating low-level exposures and their potential health risk. The approach – referred to as the "Key Events Framework" – involves a systematic examination of key biological events from initial exposure to adverse effect, including consideration of various protective mechanisms available to restore the normal physiological environment (e.g., homeostatic mechanisms, repair mechanisms, immune response). The "Key Events Framework" can be applied to a range of foodborne agents, and it provides a platform for sharing data, knowledge and new biological insights across disciplines. The proposed symposium will describe the Key Events Framework and present case studies illustrating application of this analytical framework to agents that may be present in food. A cross-disciplinary panel will discuss implications for advancing food safety, harmonizing the assessment of health risk, and facilitating the establishment of food safety standards.

S19 Round Up Your Pathogen Plan: Enrichment, Sample Preparation and the Legal and Social Perspectives

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MARK W. CARTER, Silliker, Inc., Homewood, IL, USA
BYRON F. BREHM-STECHER, Iowa State University, Ames, IA, USA
LEE-ANN JAYKUS, North Carolina State University, Raleigh, NC, USA
CRAIG K. HARRIS, Michigan State University, East Lansing, MI, USA

This symposium will discuss the current and new technologies of enrichment and sample preparation, while covering the legal and social dimensions for a pathogen-testing plan. Sample preparation after enrichment is a critical step for making food matrices suitable for many advanced detection technologies. In recent years, some proprietary media products have been launched and new technologies including bacteriophages have been developed to improve the efficiency of conventional enrichment. The development of novel approaches for sample preparation will be critical in the development of future, more rapid detection methods. The initial presentations will focus on enrichment and sample preparation, which can have the largest impact on the success of any pathogen detection plan. The concluding presentations will discuss the legal and social historical case studies related to a pathogen plan. Highlighting what is needed in an outbreak investigation, what will be needed in a court of law, and the legal ramifications of those choices. 1. New technologies and media products for rapid enrichment of pathogens in foods. 2. A new trend of industry practice: Compositing and reduced media volume – its use and impact on enrichment and rapid detection of *E. coli* O157 and *Salmonella*. 3. Extraction, concentration and purification: The "middle man" to enrichment and detection 4. Rounding them all up together: Phenotype/genotype independent target concentration 5. To Test or Not to Test: Why or Why Not to Implement a Food Safety Microbiological Testing Plan from Legal and Social Perspectives: Part I: Case Histories 6. To Test or Not to Test: Why or Why Not to Implement a Food Safety Microbiological Testing Plan from Legal and Social Perspectives: Part II.

S20 Environmental Reservoirs of Major and Emerging Foodborne Pathogens

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HUA H. WANG, The Ohio State University, Columbus, OH, USA
MARK D. SOBSEY, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA

The environment plays a significant role in harboring (persistence) of foodborne apthgens as well as facilitating their environment. The impact can be observed at both local and global levels. This symposium addresses some of teh major environmental reservoirs of food and waterborne pathogens and provides attendees with an updated overview of thier impact on foodborne diseases.

S21 Integrating Epidemiology and Microbiology to Solve Complex Food Safety Problems

RANDY SINGER, University of Minnesota, College of Veterinary Medicine, St. Paul, MN, USA
IAN GARDNER, University of California-Davis, College of Veterinary Medicine, Davis, CA, USA
LEE-ANN JAYKUS, University of North Carolina, Raleigh, NC, USA

This symposium highlights the collaborations between epidemiology and microbiology in understanding and exploring the complex issues of food safety and foodborne diseases. Epidemiology provides the scientific approach for designing longitudinal studies that help fill gaps in understanding environmental, animal, and human interactions that affect foodborne pathogens. Sampling, statistical analysis, and proper microbiologic methods are critical in the study of food safety. This symposium will focus on the integration of microbiology and epidemiology in the study of specific foodborne problems.

S22 Third Party Certification Systems: Can It Make Our Food Safer?

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DAVID ATCHISON, Dept. of Health and Human Services, Washington, D.C., USA
RENA M. PIERAMI, Silliker, Inc., Homewood, IL, USA
FRANK YIANNAS, Walmart, Bentonville, AR, USA
DONNA M. GARREN, National Restaurant Association, Washington, D.C., USA

Large national food recalls continue their progression including everything from peppers to peanut butter and it appears the food industry and government regulators are ineffective in stopping this re-occurring pattern. Consumer confidence in the ability of the food industry and government regulators to deliver a safe and wholesome food also continues to fall. All food processing plants undergo a multitude of outside third party audits based on a multitude of varying requirements, depending on the purpose of the audit. This redundancy has not been effective in reducing food

safety concerns. As a result, the food processing and food auditing industry began converting to a uniform certification program for third party audit schemes that is internationally recognized. The intent is for the various third party programs (standards) to be benchmarked against one set of principles to ensure better consistency in the application and outcome of a third party audit. This system has the ability to reduce thirty party audit duplication, reduce food safety recalls, improve consumer confidence and bridge the regulatory shortfall.

S23 A Systems Approach to Minimize *Escherichia coli* O157:H7 Food Safety Hazards Associated with Fresh and Fresh-cut Leafy Greens

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A multi-institutional team is conducting research to obtain data to enable stakeholders to assess the risk of contamination of leafy greens by *Escherichia coli* O157:H7 and identify the most influential risk management strategies. Specific goals address: (1) strategies for O157 inactivation on surfaces of compost heaps; (2) field condition influences on O157 internalization and surface contamination of leafy greens, (3) transfer of O157 during fresh-cut processing operations; (4) use of processing water as an indicator of O157 contamination; (5) processing interventions for O157 on leafy greens; (6) postharvest storage effects on O157; (7) mathematical risk modeling to identify highest risk leafy greens and impactful risk mitigation strategies; and (8) education and outreach to disseminate research outcomes and mitigation strategies.

S24 Emerging Chemical Hazards in Food

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RICHARD STADLER, Nestlé Product Technology Centre Orbe, Orbe, Switzerland
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Food companies can no longer rely on routine quality control measures to assure food safety. It is clear that emerging issues such as food packaging plasticizers migrating into foods and pharmaceuticals in water are important concerns for consumers. Not only is it important to quickly respond to emerging issues, but it is also important to resolve the issues as they arise. In addition, technology advances have resulted in the use of new substances such as nanomaterials in food applications. Alternative approaches to assess the potential hazard and exposure of these materials may be required. This symposium showcases emerging issues related to chemical hazards in food that occur at the farm through environmental pollutants (e.g., toxic metals, antibiotics or pharmaceuticals), during processing of foods (e.g., acrylamide, furan) or as a result of the migration of chemicals from packaging materials. New approaches and technologies to assess and mitigate the risks associated with these contaminants will be discussed. Initiatives taken by regulatory agencies in controlling these chemical hazards will also be presented.

S25 Food Safety Challenges for Unrefrigerated Display of Ready-to-Eat Foods

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Food Safety Challenges for Unrefrigerated Display of RTE Foods – Many retail food outlets have unrefrigerated display stations throughout their stores to entice customers into purchasing certain food products. In most cases, these display stations do not have refrigeration capability. The operation of these unrefrigerated display stations for refrigerated RTE foods generally is a violation for the Retail Food Code. This symposia will explore current industry practices and the microbiological public health challenges related to display for retail sale of refrigerated RTE foods in unrefrigerated environments.

S26 *Shigatoxin Escherichia coli:* The Bad, the Worse, and the Pathogenic

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PETER GERNER-SMIDT, CDC, Atlanta, GA, USA
JENNY SCOTT, Grocery Manufacturers Association, Washington, D.C., USA
PETER FENG, FDA, College Park, MD, USA

Shigatoxigenic *E. coli* (STEC) are of importance in food safety, having been implicated in several foodborne outbreaks worldwide for the last years. However available evidences have indicated that many STEC are not actually pathogenic to humans. What is clear is that an expanded group of STECs are present on the radar screen of public health and food regulatory authorities. The increasing concern over STEC has raised a number of questions among scientists, epidemiologists, food producers, retailers, regulators and consumers worldwide. Among the unanswered questions are: (1) What defines/differentiates STEC that are of legitimate health concern from those that should be considered benign? (2) Which food products can serve as vehicles for transmission of various pathogenic STECs to the human population?

(3) What is the perception of the food industry towards that emerging threat? (4) What methodologies are available for the detection, the isolation of these bacteria, and for the investigations of food-related outbreaks? This symposium is intended to provide attendees with an up to date understanding of this quickly evolving issue in food safety with a perspective appropriate for the expanding globalization of the food supply. International speakers are proposed in order to provide perspectives from, and to relate experiences with various STECs from regions with both apparently different endemic STEC flora and disparate farming practices.

S27 Focusing Our Efforts: Vulnerability Assessment and Mitigations Research in Food Processing and Handling Default

DONALD A. KAUTTER, FDA, College Park, MD, USA
DAVE WANKOWSKI, Kraft Foods, Glenview, IL, USA
SHAUN KENNEDY, University of Minnesota, St. Paul, MN, USA

Recent events such as melamine contamination of milk products and *Salmonella* in peanut butter reaffirm the need for increased vigilance with regard to protecting the food supply from unintentional and intentional adulteration. Food defense efforts are focused on the latter and have revealed many vulnerable points in the food supply. Vulnerability assessment (VA) is the process by which regulators and industry officials can identify these points and develop mitigations to shield them. FDA recommends CARVER+Shock as a means to conduct a VA and has developed a software program to facilitate the process. Second generation software is nearing completion along with a mitigations database to support it. Representatives from industry and academia will discuss vulnerabilities, mitigations and food defense research developments.

S28 CSI: Beverage Plant: On the Trail of Hot- and Cold-fill Spoilers

EMILIA RICO-MUNOZ, BCN Research Laboratories, Inc., Rockford, TN, USA
GORDON HAYBURN, The Tetley Group Ltd., Greenford, Middlesex, United Kingdom
WILFREDO OCASIO, The National Food Laboratory, Inc., Dublin, CA, USA
KATHLEEN A. LAWLOR, PepsiCo, Valhalla, NY, USA

An exploding package is a telltale sign that there is an issue. However, microbes do not always announce their presence so loudly. From fermentation to formula degradation to visible outgrowth, the impact of yeasts, molds, and lactic acid bacteria can be profound. The focus of this short symposium is the detection, investigation and mitigation of spoilage incidents involving high-acid beverages. The speakers will provide an update on current and future-state tools available to identify the culprits, and present a root-cause analysis of these incidents. Case studies involving microbiological contamination of hot-fill and aseptic beverages will be presented by expert investigators.

S29 Food Safety Programs across an Integrated Poultry Industry

PAULA FEDORKA-CRAY, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, USDA-ARS, Athens, GA, USA
BILLY HARGIS, Center of Excellence for Poutlry Science, University of Arkansas, Fayetteville, AR, USA
SHANE CALHOUN, Pilgrim's Pride Corporation, Pittsburg, TX, USA
SCOTT STILLWELL, Tyson Foods, Springdale, AR, USA

The modern poultry industry is different from the majority of the meat and seafood industries because of vertical integration and a business model that attempts to capture those efficiencies. Even with all parts of a complicated system under one company, there can be many challenges to defining food safety practices / interventions that are both effective in terms of public health, environmental quality, worker safety, animal health, and last but not least, profit. This short symposium brings two scientists and two poultry industry executives to you to talk about management practices or interventions from the farm to the table. Dr. Billy Hargis of the University of Arkansas will talk about challenges of controlling pathogens on the farm in the ante-mortem environment. Dr. Paula Cray from the USDA Agricultural Research Service in Athens, Georgia, will follow *Salmonella* into the slaughter plant, both with serotypes and DNA, to study antibiotic resistance factors. Mr. Shane Calhoun, Director of Microbiology for Pilgrim's Pride Poultry, will speak on interventions and management of those interventions within the slaughter environment that reduce pathogens on raw product. To tie it all up, Mr. Scott Stillwell, Vice President for Food Safety and Quality for Tyson Foods, will discuss how an integrator can take that information and science from farm to table and apply it in today's business environment.

ROUNDTABLE ABSTRACTS

RT1 Public Health Decision Making – A Character Building Exercise

Food safety agencies are often criticized for their apparent confusion and inability to act promptly to contain outbreaks. There is a general lack of understanding and appreciation for the complexities of a foodborne outbreak investigation, not only by consumers who are impacted by the outbreak, but also by regulatory authorities at local, regional and national levels who must make the decisions on appropriate actions. If hindsight were foresight we wouldn't have to deal with these issues. This interactive roundtable seeks to demonstrate to food scientists/microbiologists/epidemiologists and consumers the complexity of issues regulatory agencies face in the management of outbreaks. The roundtable discussion with relevant stakeholders (government, industry, legal and consumer) takes us through an outbreak investigation as it unfolds, explores the options available as information comes to hand, the actions agencies make and the dilemma that confronts such decision making. How much evidence is sufficient for health protection agencies to name a suspect food item? Does the need to prevent further cases outweigh the commercial viability of a company or an industry? What message are they conveying to persons affected by the outbreak? Audience participation is invited to explore issues (such as industry practice standards, capacity within the Public Health system, evidence needed to support actions taken, risk communication) that will be raised through the discussion. The food scientists/microbiologists/epidemiologists and consumers in the audience will be exposed to the myriad considerations that go into a decision to issue a public warning and to recall a suspect food product. This roundtable, through providing an insight into what happens behind the scenes, can be used to encourage better collaboration between stakeholders.

RT2 Selling Food Safety to Employees: Creating a Fully Functioning Food Safety Culture in Retail Grocery and Foodservice Operations

TODD ROSSOW, Publix Super Markets, Inc., Lakeland, FL, USA
DONNA M. GARREN, National Restaurant,Washington, D.C., USA
STEPHEN J. KENNEY, Darden Restaurants, Orlando, FL, USA
SHARON P. WOOD, H-E-B, San Antonio, TX, USA
GINA R. NICHOLSON, The Kroger Co., Columbus, OH, USA
ANN MARIE MCNAMARA, Jack In The Box, San Diego, CA, USA

Good science and broad research is key to sound practices, however, the age old question still exists regarding successful execution of best practices by the actual operators. High turn over and a range of age and experience can lend to inconsistent execution of food safety practices on a daily basis. The overriding theme of this roundtable session is creating a food safety culture that is sustained. The goal of this roundtable session is to share best practices and strategies for sustainment and maintenance of these practices that affect food safety and quality attributes. Retail foodservice operations to include grocery, restaurant, catering as well as manufacturing will benefit from this forum. The target audience for this session are those who want to take practical applications back to apply to their unique operations. Presentations will be made by experts in the field to include management and actual operator testimonials of what works. There is much science, research and emphasis on the "farm to fork" food safety applications, however, the concentrated efforts up stream in the food supply can be negated by improper execution at the retail / operator level. Best practices as well as practical, innovative and efficient tools will be shared to assist retailers / food service operations in maintaining proper execution despite work force variability and the challenges of throughput expectations. It is a vision that this roundtable would offer a spring board for a workshop to be sponsored as a follow up at the 2010 IAFP conference.

RT3 Measuring and Interpreting Food-handling Behavior and Its Impact on Policy

RANDY PHEBUS, Animal Science and Industry, Kansas State, Manhattan, KS, USA
BEN CHAPMAN, Extension, North Carolina State University, Raleigh, NC, USA
AMY SIMONNE, FYCS, Florida State, Gainesville, FL, USA
LYDIA MEDEIROS, Human Nutrition, Ohio State, Columbus, OH, USA
ROBERT BRACKETT, GMA Brands, Washington, D.C., USA

How the food preparer handles food affects the likelihood of foodborne illness. Poor washing and cleaning practices lead to gross contamination while the lack of proper temperature management can enhance microbial growth or fail to eliminate pathogens. This roundtable will document contemporary handling practices in the home and food service arena, address the challenges and successes of behavior modification, and conclude with the role of public policy to enhance safety through food supply or food handling regulations. Brainstorming between panel members and the audience as to approaches to enhance food safety will conclude the session.

D Pros and Cons of Zero-tolerance Policy for Pathogens in Food

CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, Washington, D.C., USA
EMILIO ESTEBAN, USDA-FSIS-OPHS-EALS, Athens, GA, USA
RUSS FLOWERS, Silliker Group Corp., Homewood, IL, USA

Zero-tolerance policy is in place in the United States for *E. coli* O157:H7 in ground beef and *Listeria monocytogenes* in Ready-to-Eat foods. Was the zero-tolerance policy implemented as a result of scientifically- or emotionally- or legally based concerns? Is the current zero-tolerance policy effective? What program/interventions could replace the zero-tolerance policy? Which food products should be included in a zero-tolerance policy, and which should be excluded? In this new debate-format symposium, we have gathered members from both sides to address these same four questions, followed by a debate of the issues with questions from the audience.

Special The War on Water: Cleaning for Processors of Low A_w Food

JEFF KORNACKI, Kornacki Microbiology Solutions, McFarland, WI, USA
JOE STOUT, Kraft Foods, Inc., Glenview, IL, USA
PHIL WOLFF, USDA, Washington, D.C., USA
SUZANNE TORTORELLI, Campbell's Soup, Camden, NJ, USA
LINDA HARRIS, University of California-Davis, Davis, CA, USA

Traditional cleaning methods all share a common trait. They require a lot of water. Pre-rinsing, dilution of cleaning chemical concentrates, a second rinse, and finally sanitizing with a diluted antimicrobial chemical can all be part of a typical hygiene process and they all require large quantities of water. What happens when using all that water is not an option? Perhaps the food being processed would be harmed by that much water. Maybe the water use needs to be minimized as part of a sustainability program. Or the use of that much water may result in hygiene risk on its own. Whatever the reasons for using less water, some fundamental questions need to be answered when trying to clean without water. How can pathogens be controlled or eliminated from a food processing environment that must be kept dry? Can dry cleaning eliminate allergens from food contact surfaces and how do you validate that the surfaces are safe to use? These are some of the topics that will be addressed in this symposium. In addition to addressing general questions about dry cleaning; industry, government and academic experts will provide industry specific information on dry cleaning powder milk manufacturers, bakery and cereal processors, and nut operations. Whether the motivation for dry cleaning is saving water, manufacturing the highest quality products, or improving the safety of products that have been of recent food safety concern such as nuts, this symposium will provide timely information that will be of use to a variety of different food safety professionals.

POSTER ABSTRACTS

P1-01 Accounting for Product Residue Effects when Modeling Bacterial Transfer between Processing Equipment and Meat Products

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Introduction: Cross-contamination or transfer of bacteria during handling/slicing/cutting of raw and Ready-to-Eat meats is an ongoing concern that has been identified as a knowledge gap in risk analyses. Therefore, accurate modeling of bacterial transfer during slicing/cutting is essential if quantitative microbial risk assessments for these products are to be reliable.

Purpose: The objective of this study was to quantify and model the transfer of two bacterial pathogens between equipment surfaces and meat products during sequential contact events, including slicing, accounting for the bacterial transfer effect of product residue on the equipment surfaces.

Methods: Sequential bacterial transfer experiments (*Escherichia coli* O157:H7 between beef lean or fat and stainless steel or HDPE, and *Listeria monocytogenes* between deli meats and the stainless steel blade of a commercial slicer), were conducted to generate the bacterial transfer data (log CFU/cm^2 vs. number of contacts/slices). Additionally, the weight of the slicer blade was measured after each slice to quantify the amount of product residue. The resulting data were used to: (1) develop a model for bacterial transfer to/from the equipment surface and the product and (2) assess and evaluate the effect of accumulating product residue on the transfer model.

Results: A previously developed transfer probability (log-linear) model was inadequate in modeling the transfer data, because the resulting log CFU/cm^2 vs. contact/slice curves exhibited bi-phasic tendency, with a distinctive log-linear phase (< 10 contact/slices) followed by a tailing (upward concave) phase. The duration of the first phase of the curve was product specific, decreasing with increasing fat content in the product. The log-linear equation yielded a greater ($P < 0.05$) negative slope in the first phase of the log CFU/cm^2 vs. contact/slice plots, compared to the second phase of the curve. The inflection point in the two-phase curve corresponded roughly to the point where accumulating product residue exceeded ~5 microns thickness on the slicer blade.

Significance: This improved model of sequential bacterial transfer during handling/slicing/cutting of meat products is an important step towards developing more reliable quantitative microbial risk assessments.

P1-02 Effects of Third Party Audits on Use of Food Safety Technologies and Practices in United States Meat and Poultry Establishments

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Introduction: Meat and poultry establishments have an incentive to adopt improved food safety technologies and practices to increase the safety of products produced. Establishments that are regularly audited by third party auditors may be induced to implement a higher level of technologies and practices.

Purpose: The objective of this study was to determine whether use of third party audits had a statistically significant effect on the level of food safety technologies and practices in meat and poultry slaughter and processing establishments in these areas: equipment, slaughter operations, processing operations, sanitation practices, microbiological testing, and overall.

Methods: We analyzed industry survey data from nationally representative surveys sponsored by the Food Safety and Inspection Service and conducted by RTI International in 2004 and 2005. Analysis categories included cattle slaughter, hog slaughter, and poultry slaughter establishments and processing-only establishments producing Ready-to-Eat (RTE), Not Ready-to-Eat (NRTE), and raw ground beef products. Technology and practices indexes were calculated for equipment, slaughter operations, processing operations, sanitation practices, and microbiological testing, using an algorithm developed by the US Dept. of Agriculture's Economic Research Service applied to the survey responses. Statistical significance testing was conducted to determine whether the technology and practices indexes differed depending on whether the establishment was subject to third party audits.

Results: Overall combined indexes were higher for audited than for unaudited establishments, with differences ranging from 18% for RTE establishments to 59% for poultry slaughter establishments ($P < 0.01$). Indexes for specific technologies and practices were higher for audited establishments at the 5% significance level, with the exception of sanitation practices in poultry slaughter, RTE, NRTE, and ground beef establishments. Results by establishment size and type of auditor (hired by customers or by the establishment) were mixed.

Significance: These results provide an indication of the effect of third party audits on food safety technologies and practices and thus can be used as a rationale for encouraging use of third party audits to increase the level of food protection for meat and poultry.

P1-03 Dial vs. Digital Instant Read Thermometers: Availability and Accuracy for Consumers

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Introduction: Consumers are urged to use an instant read food thermometer to ensure safely cooked ground beef patties. While a tip sensitive thermometer is often recommended by food safety professionals for measuring endpoint in ground beef patties, this message is not routinely communicated to consumers. Two types of instant read thermometers are generally available to consumers: dial types, where temperature is measured along the stem, and digital types, which are tip sensitive.

Purpose: This study investigated whether the availability of dial and digital instant read food thermometers has changed in 6 years and the accuracy of these thermometers in measuring the endpoint temperature in hamburger patties.

Methods: A survey comparing the availability of dial and digital instant read food thermometers in grocery, department, hardware, drug/variety and specialty stores was conducted in 168 stores in ten counties in Idaho and Washington State, replicating a 2001 thermometer survey. Information collected included manufacturer, model number, price, dial or digital, and instruction availability, and the results were compared to the previous survey findings. Accuracy of 3 each of the most prevalent dial and digital thermometer brands was tested against a thermocouple in freshly cooked ground beef patties to identify whether the thermometers were reliable in assessing endpoint temperature during common consumer cooking methods.

Results: The availability of instant read food thermometers in Washington and Idaho has not changed significantly in 6 years: stores carrying dial thermometers have increased from 62% to 68%, whereas digital thermometer availability has remained the same, at 46%. 76% of the stores surveyed carried instant read food thermometers. 56% of the temperature readings from digital thermometers were within 1.1°C of the thermocouple standard, while 22% of dial thermometers met this standard.

Significance: The less available digital thermometer is more successful in determining the end point temperature of hamburger patties than dial thermometers.

P1-04 Toxoplasma in Swine and Cattle

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Introduction: Toxoplasmosis is a common cause of infection in warm blooded animals, including humans. Infection generally occurs by consuming cysts in raw or undercooked meats or by accidental ingestion of oocysts shed in the feces of infected cats. Toxoplasmosis in humans can be mild but is often severe in the immunocompromised and in congenitally infected children. Consumption of pork meat is considered high risk for acquisition of toxoplasmosis. Swine, along with sheep and game animals, have been mostly associated with Toxoplasma transmission to man; however, the role of beef cattle in Toxoplasma transmission has not been fully characterized.

Purpose: The objective of this project was to better define the role of beef in Toxoplasmosis.

Methods: Fifty bovine and 62 porcine samples from the US and 200 bovine and 200 porcine samples from Peru were examined. The presence of Toxoplasma in these animals was determined by PCR and serological testing (western blot and indirect hemagglutination). Tissues and blood were collected from animals slaughtered for human consumption.

Results: Of the US bovine samples examined, 14 (28%) were positive by western blot, 12% by hemagglutination and 8% by PCR. Of the bovine samples from Peru, 40 (20%) were positive by western blot, 9 (4.5%) by hemagglutination, and 1 (0.5%) by PCR. None was positive by tissue culture. Of the US porcine samples examined, 11 (17.7%) were positive by western blot, 7 (11.3%) by hemagglutination, 10 (16.1%) by PCR and 1 (1.6%) by in vitro testing. Of the porcine samples examined from Peru, 6 (3%) were positive by western blot and 2 (1%) by agglutination. None of them were positive by PCR or by tissue culture.

Significance: Cattle and swine slaughtered for human consumption were seropositive to Toxoplasma. The parasite was also identified in these samples and thus potentially a source of human infection.

P1-05 Evaluation of Fermentation/Drying and High Pressure Processing on Viability of *Trichinella spiralis* Larvae in Raw Pork and in Genoa Salami

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Introduction: Relatively little information is known on the effectiveness of high pressure processing (HPP) when used in combination with fermentation and drying to inactivate *Trichinella spiralis* larvae in Ready-to-Eat pork products.

Purpose: Evaluate the effectiveness of HPP and fermentation/drying to inactivate *Trichinella spiralis* larvae in both infected pig muscle and Genoa salami produced with trichinae infected pork.

Methods: In part A, in each of two trials 10 gram portions (2 replicates per treatment) of fresh pig masseter muscle (ca. 3.6 log larvae/g) were pressurized with either 70,000 or 87,000 psi (483 or 600 mPa, respectively) for 0.5 to 5.0 min. In part B, Genoa salami prepared with trichinae infected pork (ca. 2.3 larvae/g of salami batter) was fermented at 20°C for 6 h and then at 27°C for 26 h before being dried at 20°C for 40 h and then at 17°C as follows: (1) for 25 days (65 mm casing; a_w 0.881 + 0.012) or 35 days (105 mm casing; a_w 0.918 + 0.007), or (2) to a target a_w of 0.920 that was attained between 11 to 17 days (65 mm casing; a_w 0.920 + 0.006), or (3) to a target a_w of 0.940 that was attained between 20 to 24 days (105 mm casing; a_w 0.939 + 0.004). After drying, four chubs in each of three trials for each treatment were post-processed pressurized at 70,000 or 87,000 psi for 1 to 12 min.

Results: T. spiralis was inactivated in masseter and Genoa salami by all treatments of fermentation/drying and/or HPP as confirmed by both microscopy and mouse bioassays.

Significance: Thus, HPP of pork muscle, as well as fermentation/drying or HPP of Genoa salami, are effective for inactivating *Trichinella spiralis* larvae.

P1-06 Microbiological Quality of Selected Ready-to-Eat Foods in Kampala City, Uganda

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Introduction: Food establishments and street food vending are rapidly growing in Uganda because of increased urbanization. The majority of people involved in this food store lack general knowledge of hygiene and food sanitation. Foods are prepared and stored under poor sanitary practices, thus compromising their safety.

Purpose: The microbiological quality of selected Ready-to-Eat (RTE) foods sold in different food establishments and by street vendors in Kampala City, Uganda was evaluated. The food establishments included canteens and takeways. Beef samosas (24), stuffed chapattis (rolex) prepared at the roadside (12), and roast chicken (32) were analyzed.

Methods: Standard methods were used to determine aerobic plate counts of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. and coliform counts.

Results: The Ready-to-Eat samosas had as high as > 10^5 aerobic counts. Coliforms in samosas ranged from 1.22 ± 0.40 to 2.90 ± 0.9 log CFU/g. *Staphylococcus aureus* counts in samosas varied between 2.93 ± 0.9 and 3.58 ± 1.3 log CFU/g. Meat samosas sold in canteens had counts that were significantly ($P < 0.05$) higher than those of takeways. No significant differences ($P > 0.05$) in counts were observed between meat samosas from takeways and those of street vendors. *Escherichia coli* counts in rolex varied between 1.91 ± 042 and 3.55 ± 018 CFU/g, whereas *Staphylococcus aureus* counts ranged from 0.14 ± 042 to 1.39 ± 064 CFU/g. No *Salmonella* spp. were detected. Coliforms in roast chicken ranged from 1.73 ± 0.10 to 2.18 ± 0.03 log CFU/g, whereas *Staphylococcus aureus* varied between 1.56 ± 0.00 and 2.43 ± 0.02 log CFU/g. The microbial numbers were significantly ($P < 0.05$) higher in roast chicken from street vendors than in those from takeways.

Significance: It was concluded that RTE foods analyzed in this study were of unacceptable microbial quality and could lead to foodborne illnesses as a result of poor handling practices and hygienic conditions prevailing in the food establishments.

P1-07 Applying a Path-dependent Model for *Salmonella* Thermal Inactivation in Slow-cooked Turkey and Beef Products

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Introduction: Thermal process lethality is currently determined in industry using models that predict inactivation rate based solely on the present state of the food product. However, it is known that sub-lethal injury, which might occur during slow heating processes, can increase the thermal resistance of bacteria.

Purpose: The objective of this project was to estimate the parameters for a modified 1st-order-Arrhenius inactivation model, which accounts for the effect of prior sub-lethal heating on subsequent inactivation rates, and to test the model against *Salmonella* inactivation in meat products cooked in a moist-air convection oven.

Methods: Irradiated (>10 kGy) ground turkey breast and beef round were inoculated with an 8-serovar *Salmonella* cocktail, and 1-g samples were subjected to 23 different non-isothermal heating profiles in a thermocycler to target lethalities of 3-5 log reductions (in triplicate). Each heating profile consisted of a randomly selected combination of a linear heating rate (1, 2, 3, 4, or 7 K/min), a variable-length sub-lethal holding period (40, 45, or 50°C), and a final holding temperature (55, 58, 61, or 64°C). Survivors were enumerated on aerobic Petrifilm™. Sub-lethal injury (τ) was quantified as an integral function of the temperature profile between 38 and 52°C. The three parameters of the new model were estimated via non-linear regression of computed vs. experimental survivor data. Additionally, 25-g, inoculated samples (n=9) of the same products were cooked in a computer-controlled, laboratory-scale, moist-air convection oven, with variable cooking schedules (30-120 min) to a target 7.0 or 6.5 log reductions for turkey and beef, respectively.

Results: The error between the traditional, state-dependent model predictions and experimental lethality increased ($P < 0.05$) with τ, with fail-dangerous deviations as high as 8 and 7 log CFU/g for turkey and beef, respectively, in the 1 g samples. However, the systematic error with τ was eliminated ($\alpha = 0.05$) when the new model was applied. For the oven-cooked product, the traditional model significantly ($P < 0.05$) over-predicted *Salmonella* lethality, with errors as high as 3 log reductions increasing with τ. However, when the new model was applied to the same data, the root mean squared error was reduced from 2.4 log to 0.7 log.

Significance: The results show that significant process lethality errors can occur in slow cooked meat products, which could jeopardize product safety; however, a new, path-dependent inactivation model eliminated the systematic error.

P1-08 Behavior of *Salmonella* spp. in Ground Beef Containing Sodium Lactate and Oregano during and after Heat Treatments

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Introduction: Inadequate time and temperature combinations are often employed for cooking some ground meat products, such as hamburger patties, for sensory reasons. Pathogens may survive in such undercooked products and can result in food poisoning outbreaks.

Purpose: The objectives of this study were to assess the efficacy of sodium lactate (NaL) and oregano in inactivating *Salmonella* spp. during heat treatments and controlling subsequent growth of the surviving bacterial cells during storage.

Methods: A cocktail of 8 strains of *Salmonella* spp. was inoculated into ground beef supplemented with NaL (1.5% and 3.0%) and oregano (0.5% and 1.0%) to obtain ~8.0 log CFU/g. The ground beef samples (3 g) were vacuum-packed and

heated at 60, 65, or 71°C in a circulating water bath for selected times to inactivate ~5.0 to 6.0 log CFU/g of the pathogen, and then stored at 15°C for 15 days. The inactivation rates during heating and the fate of *Salmonella* spp. during storage were determined.

Results: In general, the addition of oregano and/or lactate did not affect the inactivation rates at 60 and 65°C when compared to control samples. The inactivation rates with 3.0% lactate, 3.0% lactate + 0.5% oregano, and 3.0% lactate + 1.0% oregano supplemented in ground beef and heated at 71°C were 9.1, 12.8, and 12.9 log CFU/min, respectively. These inactivation rates were all significantly higher ($P < 0.05$) than the control (6.8 log CFU/min). Oregano or lactate showed a growth inhibitory effect against *Salmonella* spp. during storage, particularly in samples treated at 65 and 71°C. At 65°C, no growth were observed in ground beef containing 1.0% oregano with or without lactate, and at 71°C growth was not observed in any samples, regardless of the presence or absence of lactate or oregano.

Significance: Results indicate that lactate and oregano may be used to render *Salmonella* spp. more sensitive to the lethal effect of heat and to inhibit growth of the pathogen that survive the heat treatments.

P1-09 Accuracy of Interval Accumulation-based Tools in Predicting Behavior of *Staphylococcus aureus*, *Salmonella* serovars, and *Escherichia coli* O157:H7 in Pork Products during Single and Repeated Temperature-abuse Periods

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Introduction: The THERM (Temperature History Evaluation of Raw Meats) tool for predicting growth of *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* O157:H7 uses linear interpolation of experimentally determined lag-phase duration and growth rate data in an interval-accumulation technique to make predictions based on an entered time/temperature history. It is not known how accurately THERM will predict pathogen behavior in a wide range of raw pork products, particularly when these products are temperature-abused, returned to temperature-control, and then temperature-abused again.

Purpose: The objective of this study was to compare THERM predictions to the observed behavior of *Staphylococcus aureus*, *Salmonella serovars*, and *Escherichia coli* O157:H7 in a range of inoculated pork products exposed to single and sequential temperature-abuse conditions.

Methods: Inoculation studies were done with cooling of warm pork sausages, warming of pork bratwurst, isothermal temperature-abuse of pork frankfurter batter, and two sequential periods of 13°, 15.6°, or 21.1°C temperature-abuse of pork breakfast sausage, natural pork chop, and enhanced pork loin, separated by refrigeration (5°C, 24 h) or freezing (-20°C, 24 h) and thawing (24 h, 5°C).

Results: Pathogen-growth predictions from THERM developed using ground pork and sausage were compared to experimental results of 0 to 3.0 log CFU of growth. Across all temperature-abuse conditions, qualitative (growth vs. no-growth) predictions made using the pork tool (n = 115) and the sausage tool (n = 133) were, respectively, accurate (51%, 50%), fail-safe (44%, 50%), or fail-dangerous (5%, 0%). Quantitative predictions from the two tools were, respectively, accurate (29%, 22%), fail-safe (59%, 73%), or fail-dangerous (12%, 5%). Pathogen growth was greater during the second sequential temperature-abuse period, but not significantly so ($P > 0.05$).

Significance: THERM tools provide accurate-to-fail-safe predictions of pathogen behavior in raw pork products subjected to a range of temperature-abuse conditions, and will be useful in supporting post-deviation corrective actions.

P1-10 DSC Inactivation Kinetics of a Four-strain Composite of *Salmonella* Enteritidis and Oranienberg in Commercially-acquired Liquid Egg Yolk

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Introduction: Current liquid egg pasteurization requirements are based on command and control prescribed time/temperature combinations for specified egg products. Requirements are found in the Code of Federal Regulations, Title 9, Ch. III, Sec. 590.570. These standards were based on data for the inactivation of *Salmonella* in liquid egg products acquired prior to 1970 and are currently being reevaluated in light of recent risk assessments, which take into account changes in industrial practices such as in-line egg processing and variation in egg product formulations not represented in the current regulation.

Purpose: The goal of this study was to determine the inactivation kinetics of a four-strain composite of thermally-resistant strains of *Salmonella* inoculated into commercially-processed liquid egg yolk.

Methods: Heat-resistant *Salmonella* (three serovars of Enteritidis [phage types 8, 8, and 13] and one Oranienburg) were selected for resistance to 50 µg/ml nalidixic acid. Cultures were grown to stationary phase in Tryptic Soy Broth at 42°C, with one 24 h transfer, concentrated ten-fold by centrifugation, and resuspended in 0.1% peptone water. Each inoculum was added to liquid egg yolk and mixed thoroughly, resulting in a final population of ca. 8 log CFU/ml egg yolk. Inoculated yolk was injected into sterile glass capillary tubes, flame-sealed and heated in a water bath at 58, 60, 62, 64, and 66°C. Capillary tubes were ethanol sanitized and rinsed twice in sterile water, and contents were extracted. Yolk was diluted, surface plated onto Tryptic Soy Agar + 0.1% sodium pyruvate and 50 ug/ml nalidixic acid and incubated at 37°C for 24 h before colonies were enumerated.

Results: Decimal reduction values were calculated from survivor curves with a minimum inactivation of 6 log CFU/ml at each temperature. The asymptotic D values were 2.06 min at 58°C, 0.82 min at 60°C, 14.4 s at 62°C, 5.73 s at 64°C and 2.15 s at 66°C.

Significance: These results provide useful information that will be used by the USDA Food Safety Inspection Service for issuing new pasteurization log reduction performance standards and industry guidance rather than command and control temperature requirements.

Poster

P1-11 Evaluation of Microwave Cooking Procedures for Frozen, Raw, Breaded Chicken Products to Ensure *Salmonella* Inactivation

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Introduction: Consumption of frozen, pre-browned (but raw), single-serving stuffed chicken products prepared in the home has led to several confirmed salmonellosis cases recently. Products appear fully cooked and package labels do not always effectively inform consumers of proper preparation procedures. The USDA-FSIS has advised processors to validate cooking instructions and modify package labels to state that a minimum 165°F internal temperature must be achieved. In an affiliated study, consumers were observed preparing representative products according to label instructions, with frequent variations in microwaving procedures being witnessed.

Purpose: This study evaluated microwave cooking label instructions, with observed consumer procedural variations, for their ability to inactivate high levels of *Salmonella*.

Methods: Slightly thawed chicken cordon bleus, chicken kievs and chicken strips were inoculated internally (6 to 7 log CFU/g) with a 5-serovar mixture of *Salmonella* and held at -20°C. Each product was cooked as single (for 4 min) or double (for 7 min) units in 600 or 1000 W microwave ovens with turntables. Treatment variations included mid-treatment flipping and/or use of a plastic microwavable cover. Internal temperature profiles for all products and corresponding *Salmonella* reductions were determined.

Results: For all products studied, highly variable internal temperatures were recorded across individual samples in 600 W ovens for both single and double product units. In virtually all 600 W samples, cold spots were determined, with samples demonstrating temperatures as low as 93°F and *Salmonella* reductions ranging from 1 to 6 log cycles. All 1000 W cooked samples achieved internal temperatures of ≥ 165°F and were *Salmonella* negative by enrichment detection, with the only exception being single and multiple units that were neither flipped nor covered during cooking. This treatment demonstrated low-level survival after enrichment.

Significance: Microwave cooking of frozen, raw breaded poultry products is unpredictable in achieving uniform target end-point temperatures; however, ovens with wattages ≥ 1000 provide substantially reduced pathogen survival risks if validated cooking instructions are followed.

P1-12 Influence of Jamaican Jerk Seasoning Paste on Growth of Natural Bacterial Flora and *Salmonella* Typhimurium on Raw Chicken Breast Meat

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Introduction: Jamaican jerk seasoning paste is a unique blend of herbs and spices (scallions, scotch bonnet peppers, thyme, nutmeg, allspice) and citric or acetic acid. Seasoning components such as thyme, allspice and nutmeg are known to have antimicrobial properties. Although Jamaican jerk seasoning is used to add flavor to various meat dishes, there is no published research describing its antibacterial effects in meats.

Purpose: This research was conducted to evaluate the antibacterial effect of two commercial brands of Jamaican jerk seasoning paste (GRA and WAL) on raw chicken breast meat at 4°C and 25°C.

Methods: Seasonings were applied to portions of raw, skinless, boneless breast meat at concentrations of 3.2%, 4.8%, and 6.4% (w/w). Meat without added seasoning served as control. Samples were aerobically packaged and refrigerated (4°C) for 6 days. Separate portions of control and seasoned meat were inoculated with a nalidixic-acid-resistant strain of *Salmonella* Typhimurium ATCC 14028 and held aerobically at 25°C for 8 h. At set time intervals numbers of total aerobic bacteria, Enterobacteriaceae, and *Salmonella* were evaluated by serially diluting (10-fold) swab samples from 10 cm^2 areas of meat followed by plating diluted samples on appropriate agar media.

Results: Populations of aerobic bacteria in control (4°C) increased rapidly from ~5.43 log CFU/cm^2 and reached 7.42 to 7.93 (day 3) and 8.78 to 9.2 log CFU/cm^2 (day 6). Generally, bacterial growth inhibition increased with increasing seasoning concentration. Jerk seasoning (6.40%) strongly inhibited bacterial growth with viable counts remaining at ~ 5.34 (WAL) and 5.16 (GRA) log CFU/cm^2 up to 4 days. A similar trend in inhibition of Enterobacteriaceae was observed. In seasoned meat at 25°C, *Salmonella* did not grow for up to 4 h irrespective of seasoning brand or concentration. Both brands of seasoning initially lowered surface pH of the meat by 0.15 to 0.41 units.

Significance: Based on these results, certain brands of Jamaican jerk seasoning paste are effective in delaying microbial spoilage of raw chicken meat at 4°C; however, use of seasonings at 3.2 to 6.4% may not prevent the growth of *Salmonella* on the meat after 4 h of temperature abuse (25°C).

P1-13 Reduction in *Salmonella* Positives and Microbial Counts on Chicken Carcasses Treated with 360 to 1800 ppm Peracetic Acid, Using Spectrum™ in the Finishing Chiller to Achieve USDA Category 1 Status

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Introduction: Safety of food products from commercial production facilities is always a concern, especially in light of recent events highlighted in the media concerning public health. In addition, increasing regulatory pressure and tightening limits on *Salmonella* levels in poultry presents a challenge to processors. Antimicrobial interventions, such as the use of peracetic acid (PAA) (Spectrum™, FMC Corporation), are used to meet the *Salmonella* criteria set forth by the USDA (< 10% positive for Category 1). Spectrum™ can currently be used for pathogen control at 25 to 200 ppm PAA.

Purpose: This study was conducted to assess the antimicrobial efficacy of Spectrum™ between 360 and 1800 ppm PAA to control *Salmonella* and reduce microbial counts, using only a finishing chiller for antimicrobial intervention at a commercial poultry production facility.

Methods: Over 400 chicken carcasses were sampled using the FSIS bird rinse methodology. Carcasses exposed to levels between 360 and 1800 ppm PAA in a commercially available finishing chiller, and baseline carcasses chilled in totes without PAA were tested over a total of 4 days during 2 separate weeks to account for flock to flock variability. Due to the higher levels of PAA in the test, sodium thiosulfate was added to the rinsate in order to neutralize any residual prior to testing. Samples were tested using 3M™ Petrifilm™ for aerobic plate count, coliforms/*E. coli*, and for *Salmonella* using BAX® PCR (DuPont Qualicon; Wilmington, DE).

Results: Aerobic plate counts were reduced from 3.41 to 5.19 log colony forming units (CFU) per mL of rinsate in the baseline samples to 2.3 log (360 ppm PAA), 1.01 log (500 ppm), 0.32 log (675 ppm), 0.35 log (900 ppm), 0.78 log CFU/mL (975 ppm) and below detection (1800 ppm). Likewise, *Salmonella* was reduced from up to 90% positive in the controls to 0% positive for all levels of PAA except 975 ppm, which was found to be 6% positive (84% reduction vs. control at this test level). No organoleptic changes were observed in the treated carcasses.

Significance: Spectrum™ PAA at use concentrations between 360 and 1800 ppm provided significant reduction in microbial recovery and *Salmonella* positives. At 1800 ppm, it reduced the microbial recovery to below detection limits, with no observed organoleptic issues. Use of Spectrum™ at these concentrations in finishing chillers will enable poultry processors to produce microbially safer products and meet USDA Category 1 status for *Salmonella*. Furthermore, due to the extremely low microbial recovery at these levels, the product shelf life may potentially be increased. FMC Corporation currently seeks FDA approval for higher use rates of Spectrum™.

P1-14 DSC Inhibition of Growth of *Escherichia coli* O157:H7 and *Salmonella* in Ground Beef Using Modified Atmosphere Packaging Systems

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Introduction: Ground beef is commonly linked to *E. coli* O157:H7 and *Salmonella* outbreaks in the USA. The use of MAP increases the quality and shelf life of meat products. However, MAP products can still be temperature abused within the cold chain, allowing pathogen growth and increasing consumer risk.

Purpose: The objective of this study was to determine if *E. coli* O157:H7 and *Salmonella* can be inhibited under consumer temperature abuse in MAP packaging environments compared to traditional PVC overwrap over a 24-day shelf life.

Methods: Multiple strains of *E. coli* O157:H7 or *Salmonella* was used to inoculate (1×10^3 CFU/g) ground beef patties (four replications). The packaging treatments were: overwrap, vacuum, high-oxygen (HO)(80%O2/20% CO_2), low-oxygen (CO), CO blend (0.4% CO/35% CO_2/64.6% N_2) , and low-oxygen (WOCO) blend (35%CO_2/65% N_2). Packages were stored at 37°F in the dark until processing. On day 6, packages were randomly placed in three temperature conditions (37°F -control), 8 h at 70°F, and 95°F for 4 h. After temperature abuse, packages were placed in a 37°F until sampling on days 6, 9, 11, 14, 24. *Salmonella* samples were plated onto Rambach agar and *E. coli* O157:H7 on MacConkey agar. The data was analyzed using descriptive analysis in SAS.

Results: There were no significant interactions. *E. coli* O157:H7 was lower in ground beef packaged WOCO and CO than the other three packaging types regardless of sampling day or temperature abuse ($P < 0.05$) Vacuum packaged samples had significantly less *E. coli* O157:H7 than the traditional overwrapped samples. The effects of the packaging type and temperature abuse were not significant ($P > 0.05$) in beef patties treated with *Salmonella*.

Significance: This study indicates that the use of MAP may be inhibitory to *E. coli* O157:H7 during temperature abuse, therefore having a positive food safety impact in ground beef.

P1-15 Heat and Acid Resistance of *Escherichia coli* Biotype I Used as Surrogates for *Escherichia coli* O157:H7 and *Salmonella* in the Validation of Pathogen Interventions in Beef Carcasses

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Introduction: Non-pathogenic bacteria isolated from environments that are common to *E. coli* O157:H7 and *Salmonella* may be used as surrogates for these pathogens in validating and verifying the effectiveness of intervention strategies designed to control hazards within a HACCP plan for beef carcasses, provided that these surrogates show a response to the factors involved in these strategies similar to that of the pathogens.

Purpose: To compare the resistance to heat and acid of various isolates of *E. coli* biotype I obtained from beef carcasses, *E. coli* O157:H7 and *Salmonella* spp.

Methods: Each of 26 isolates of *E. coli* biotype I, 7 of *E. coli* O157: H7 and 11 of *Salmonella* in stationary phase (12 h) in TSB was suspended in 10 mL of Butterfield buffer solution (pH 7.0) and heated at 55°C for 20 min and at 65°C for 10 min to determine heat resistance, and exposed to L-lactic acid solutions at pH 2.0, 3.0 and 4.0 to determine acid resistance at 35°C. At intervals during each treatment, samples were separated and tested for counts of these microorganisms by surface spreading on Tryptic Soy Agar (TSA) and TSA 4% NaCl.

Results: The D-values for *E. coli* biotype I at 55°C and 65°C ranged from 8.5 to 40.8 min and 1.9 to 3.4 min, respectively. D-values for *E. coli* O157:H7 and *Salmonella* spp. ranged from between 7.2 to 24.5 min and 1.8 to 3.4 min and between 4.3 to 10.5 min and 1.7 to 2.7 min, respectively. The reduction of *E. coli* biotype I, *E. coli* O157:H7 and *Salmonella* was increased at 65°C. Mean log reductions at pH 2.0, 3.0 and 4.0 were 5.4, 2.6 and 0.5 log CFU/ml for *E. coli* biotype I; 5.8, 2.5 and 0.6 log CFU/ml for *E. coli* O157:H7; and 5.5, 3.5, 1.3 log CFU/ml for *Salmonella* respectively.

Significance: Resistance to heat and acid of the *E. coli* biotype I isolates was higher or equal to that of *E. coli* O157:H7 and *Salmonella*. Therefore, *E. coli* biotype I may be a good index of these pathogens in validating and verifying methods for beef carcass decontamination.

Poster

P1-16 DSC Evaluation of Brining Ingredients and Antimicrobials for Effects on Thermal Destruction of *Escherichia coli* O157:H7 in a Meat Model System

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Introduction: Brine injection is used to increase palatability of lower-value meat cuts. *Escherichia coli* O157:H7 may become internalized during this process, and may result in foodborne illness if the product is undercooked.

Purpose: This study evaluated the potential effect of brining ingredients, antimicrobials and fat content on *E. coli* O157:H7 in a ground beef model system after simulated brining, storage, and cooking.

Methods: Fresh beef knuckles (5.3 ± 2.4% fat) or beef shoulder (15.3 ± 2.2% fat) were ground individually, inoculated (7.2 ± 0.1 log CFU/g) with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7, and mixed with brining solutions (to simulate a 10% pump rate). Treatments (700 g batches) included no brining, distilled water (DW), sodium chloride (NaCl, 0.5%), sodium tripolyphosphate (STP, 0.25%), NaCl + STP, and the NaCl + STP combination with added potassium lactate (PL, 2%), sodium diacetate (SD, 0.15%), PL + SD, lactic acid (0.3%), acetic acid (0.3%), citric acid (0.3%), Nisaplin® (0.06%) or pediocin (ALTA™ 2341; 0.5%) + EDTA (20 mM), AvGard® XP (0.2%), cetylpyridinium chloride (CPC, 0.5%), and hops beta acids (0.00055%). Also, sodium pyrophosphate (0.25%) was tested singly and in combination with NaCl. Samples (30 g in test tubes) were analyzed for the pathogen (Tryptic Soy Agar plus 0.1% sodium pyruvate and 100 µg/ml rifampicin) immediately after mixing, storage (24 h at 4°C), and cooking (65°C). Data (2 replications, 3 samples/treatment/replication) were analyzed as a randomized block factorial design, using the General Linear Model and Tukey's Honestly Significant Differences procedures of SAS.

Results: The effect of the fat level of the meat on microbial counts was negligible. Following 24 h of storage at 4°C, pathogen numbers in CPC-treated samples were reduced by approximately 1 log cycle, whereas for all other treatments counts remained unchanged ($P > 0.05$). Cooking of stored samples reduced counts by 1.5 to 2.5 log CFU/g. Surviving populations of *E. coli* O157:H7 in cooked samples were the lowest ($P < 0.05$) in those treated with CPC (3.7 to 3.8 log CFU/g), whereas for all other treatments, pathogen survivors (4.7 to 5.7 log CFU/g) were similar ($P > 0.05$) to the DW-control (5.1 to 5.3 log CFU/g). *E. coli* O157:H7 populations in cooked samples treated with Nisaplin® or ALTA™ 2341 were lower ($P < 0.05$) than in those treated with AvGard® XP, and PL and/or SD.

Significance: These data should be useful in development/improvement of brines for control of *E. coli* O157:H7 in moisture-enhanced meat products.

P1-17 Implementation of Multiple *Escherichia coli* O157:H7 Antimicrobial Interventions in Very Small Beef Processing Facilities

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Introduction: Beef harvesting plants now utilize antimicrobial intervention strategies to reduce and prevent carcass bacterial contamination. Multiple antimicrobial interventions have been validated to be more effective at reducing the occurrence of *E. coli* O157:H7 contamination on carcasses processed in large slaughter facilities. However, most small and very small business beef harvesting plants use one antimicrobial intervention for the control of *E. coli* O157:H7.

Purpose: This study evaluates the effectiveness of harvesting procedures involving multiple antimicrobial interventions versus a single antimicrobial intervention for the control of *E. coli* O157:H7 in very small beef processing facilities.

Methods: A total of 150 beef carcasses were evaluated across three very small meat processing facilities, 75 control (C) and 75 multiple (M) intervention carcasses. The C treatment consisted of one lactic acid (LA) (3.0%v/v) wash at the end of slaughter, just prior to chilling. The M intervention treatment received a LA (3.0%v/v) wash prior to evisceration, a hot water wash (avg. = 78.4°C) after carcass splitting and trimming, and a final LA wash just prior to chilling. Carcasses were sponge sampled for Aerobic Plate Count (APC), generic *E. coli* (EC), coliforms (CL), and Enterobacteriaceae (EB), according to USDA procedures. The inside round, hindshank, and foreshank were sponge sampled for *E. coli* O157:H7 (~4000 cm^2).

Results: Log counts (CFU/cm^2) for APC, EC, CL, and EB were similar ($P > 0.05$) for C and M carcasses before interventions were applied. Reductions in APC (CFU/cm^2) were greater ($P \leq 0.05$) for M carcasses than for C carcasses (1.42 CFU/cm^2 and 0.91 CFU/ cm^2, respectively) at the end of the harvesting process prior to chilling. Log reductions for EC, CL, and EB were also greater for M carcasses. However, log reductions for M and C carcasses were similar $P > 0.05$) after carcass chilling for APC, EC, CL, and EB. Pre-intervention C and M carcasses tested 17.3% and 18.6% positive for *E. coli* O157:H7, respectively. After chilling C and M carcasses tested 2.67% and 1.3% positive for *E. coli* O157:H7, respectively.

Significance: The M intervention treatment was more effective at reducing microbial contamination during the harvesting process. Both treatments were effective at reducing the occurrence of *E. coli* O157:H7.

P1-18 Plant Variation in the Validation of a Hot Water Antimicrobial Intervention during Harvesting Beef Carcasses in Small and Very Small Meat Processing Plants

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Introduction: Small and very small meat processors have implemented antimicrobial interventions during harvesting beef. Most plants use published peer reviewed journal articles as supporting documentation, however, very small plants are being asked by regulatory agencies to conduct in-plant validation studies. In-plant studies present challenges to small plant operators.

Purpose: The objective of this study was to determine if standard operating procedures for antimicrobial interventions during harvesting beef can be validated to reduce variation in achieving food safety.

Methods: Validation studies were conducted in 17 very small processing plants from North Carolina, Kentucky, Kansas, and Nebraska. Plants were instructed to apply a hot water intervention of 60°C or greater. Carcass sides were sampled at each plant for Aerobic Plate Count (APC), *E. coli*, coliforms, Enterobacteriaceae, and *E. coli O157:H7* according to standard procedures. The statistical analysis was designed to determine the contribution of plant as a random effect to a model that included state as a fixed effect to determine the contribution of plant to variability. The estimate for plant effect was tested as either greater than or lower than ($P < 0.05$) the estimates for other plants.

Results: Mean intervention hot water temperature was 63°C and application time 2.35 m per side. APC averaged 2.17 CFU/cm^2 prior to interventions, 1.74 log CFU/cm^2 after intervention, and 1.00 log CFU/cm^2 after chilling. Plant effects were significant ($P < 0.05$) for all microbiological measures; APC, coliforms, generic *E. coli*, and Enterobacteriaceae. State was not significant ($P > 0.05$) for most microbiological measures. Plant effects were also significant ($P < 0.05$) for estimates of reduction in log CFU/cm^2 of APC, coliforms, generic *E. coli*, and Enterobacteriaceae for the hot water intervention, chilling of the carcass, and the hot water and chilling combined. Two plants had greater ($P < 0.05$) log reductions and also had greater estimates for bacterial counts. Four plants had lower ($P < 0.05$) log reduction estimates for at least one of the microbial measures and log reduction measures. No positive samples for *E. coli* O157:H7 were found at pre evisceration or post chilling.

Significance: There was variation among small plants for the number of bacteria on the carcass during harvesting and the log reduction of bacteria on the surface of the carcass from a hot water intervention and chilling. Even though standard operating procedures were established, in-plant validation studies may be important to establish the effectiveness of an antimicrobial intervention.

P1-19 Effect of Ozone and Ultraviolet Irradiation Treatments on *Listeria monocytogenes* Populations in Chill Brines

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Introduction: Recirculated chill brines are used in Ready-to-Eat meat manufacture to rapidly cool product after a cooking step. Since recirculated chill brines may be used continuously for many days or weeks, *Listeria monocytogenes* in these brines may present a significant, ongoing post-processing contamination hazard.

Purpose: This study was conducted to evaluate the combined effect of ozone and ultraviolet light on *L. monocytogenes* populations in freshly prepared brine and spent brine collected at the end of use.

Methods: Fresh brine (9% NaCl) was prepared in the laboratory. Spent brine (20.5% NaCl; collected at the end of use) was obtained from a Ready-to-Eat meat manufacturer. *Listeria monocytogenes* (strains: N1-227, N3-031, and R2-499) were grown in TSBYE at 35°C for 24 h, washed, resuspended in fresh and spent chill brines, and combined in equal proportions. The brines were then subjected to ozonation for 5 and 10 min (fresh brine) or 30, 45, 60 min (spent brine). Following ozonation, the inoculated brines were subjected to ultraviolet light (peak output: 254 nm) in a bench-top photoreactor for 5 and 10 min(fresh brine) or 10 min only (spent brine). After the ozone and UV treatments, 1 ml portions of each brine were serially diluted in 0.1% peptone and surface-plated onto TSAYE, followed by incubation at 35°C for 48h.

Results: In fresh brines (transmittance at 254 nm = 91.8%), ozonation for 5 min followed by UV exposure for 5 min resulted in a 5.7 log reduction of *L. monocytogenes*. Whereas 10 min of ozonation followed 5 min of UV resulted in an 7.8 log reduction. In contrast, reductions of *L. monocytogenes* in spent brines (transmittance at 254 nm = 0.0%) treated by ozonation for 30 min followed by UV for 10 min were less than 0.5 log CFU/ml. Ozonation of spent brines for 60 min followed by 10 min UV exposure resulted in a 5.4 log reduction in *L. monocytogenes*.

Significance: Reduction of *L. monocytogenes* in chill brines is significantly affected by the nature of the brine solution (i.e., fresh vs spent) and the method of treatment (i.e., ozone vs UV).

P1-20 Addition of *Carnobacterium maltaromaticum* CB1 to Vacuum-packaged, Sliced Processed Meats Inhibits the Growth of *Listeria monocytogenes*

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Introduction: Listeria monocytogenes is a major concern in the safe production of processed vacuum-packaged meats. Failure to control *Listeria* on meat products has resulted in major outbreaks of listeriosis.

Purpose: The purpose of this investigation was to determine the ability of *Carnobacterium maltaromaticum* CB1 to control the growth of *L. monocytogenes* on three types of processed meats: ham, turkey and roast beef.

Methods: The sliced meats were inoculated with approximately 10^3 CFU of a cocktail of four strains of *L. monocytogenes* per gram of meat and with 10^4 CFU of live cells of *C. maltaromaticum* CB1 per gram of meat before vacuum packaging and storage at 4°C. Three replicates of each product were tested over the shelf life (from 35 to 62 days) in duplicate for each treatment and *L. monocytogenes* were enumerated on PALCAM agar with supplement.

Results: In the absence of *C. maltaromaticum* CB1, *L. monocytogenes* grew to 10^8 CFU/g. However, in the presence of CB1, numbers of *L. monocytogenes* remained at the initial inoculum level (10^3 CFU/g) on the meats. The growth rate of CB1 on the meat products differed, with a maximum population reached in 27 days on ham, 18 days on turkey and 14 days on roast beef. *Listeria* alone reached a maximum population after 27 days, 33 days and 21 days, respectively. In contrast, *Listeria* in the presence of CB1 remained at the inoculum level for 62 days, 56 days and 49 days of storage at 4°C on ham, turkey and roast beef, respectively.

Significance: The research indicated that on these three markedly different meat types, in the presence of the lactic acid bacterium *C. maltaromaticum* CB1, the growth of *L. monocytogenes* was inhibited throughout the storage life of the products. Bacteriocin-producing lactic acid bacteria, such as *C. maltaromaticum* CB1, can be used as an effective alternative to chemical interventions to stop the growth of *L. monocytogenes* on a variety of sliced processed meats.

P1-21 Modeling Elimination of *Listeria monocytogenes* from Ready-to-Eat Cooked Meats Using High Pressure Processing

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Introduction: *Listeria monocytogenes* is of great concern to the Ready-to-Eat (RTE) meat industry because of its ability to grow at low temperatures. High pressure processing (HPP) offers an alternative to ingredient-based preservation for assuring food safety of RTE meat products through its ability to reduce the numbers of *Listeria* present after packaging.

Purpose: The purpose of this study was to demonstrate the capacity of HPP to achieve complete elimination of *L. monocytogenes* from RTE meats and to model the lethality of different process conditions to facilitate process setting.

Methods: A five-strain cocktail of *L. monocytogenes* inoculated at 10^6, 10^4 and 10^2 CFU/ml into tryptone soya broth (with NaCl at 1% and 2% w/v and sodium lactate at 0% and 2.5% w/v) was pressure treated at 450, 600, 650 or 700 MPa. Each treatment was applied to sets of 5 individual tubes for different periods of time. Following pressure treatment, the contents of the tubes were tested for viability, using a modification of the USDA method. The shortest time when no tubes had surviving *Listeria* was defined as the time to inactivation (TTI). TTI was modelled as a function of the independent variables and the model was validated against RTE meat samples.

Results: For every treatment at least one set of 5 tubes showed no survivors. TTI varied with inoculum level and inversely with pressure. Lactate and NaCl concentrations did not significantly affect TTI over the range tested. *L. monocytogenes* at 10^4 CFU per pack was eliminated from sliced turkey and chicken in < 5 min. Observed TTI was in line with predictions from the model.

Significance: The data confirm that high pressure processing is able to eliminate *L. monocytogenes* from RTE meats with the required process pressure and hold times dependent on the initial level of *Listeria* present. A process of 600 MPa for 5 min was shown to be capable of eliminating 10^4 *L. monocytogenes* per package of meat.

P1-22 Effectiveness of Fermentation/Drying and Post-process Pressurization on Viability of *Listeria monocytogenes* and *Salmonella* spp. in Genoa Salami

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Introduction: There are a number of physical and chemical interventions that are used as post processing interventions to control pathogens in Ready-to-Eat meats.

Purpose: Evaluate the effectiveness of fermentation/drying, and high pressure processing (HPP), in inactivating *L. monocytogenes* or *Salmonella* spp. (ca. 7.0 log per gram of each in batter) in Genoa salami.

Methods: Inoculated chubs were fermented at 20°C for 6 h and 27°C for 26 h and then dried at 20°C for 40 h and 17°C for: (1) 25 days (65 mm casing; a_w 0.881) or 35 days (105 mm casing; a_w 0.918), or (2) to a target a_w of 0.920 that was attained between 11 to 17 days (65 mm casing; a_w 0.920), or (3), to a target $a_w$of 0.940 that was attained between 20 to 24 days (105 mm casing; a_w 0.939). After drying, four chubs for each treatment were pressurized at 70,000 (483 mPa) or 87,000 (600 mPa) psi for 1 to 12 min. Next, some chubs were sampled for the pathogen while others were vacuum sealed and stored at 4°C.

Results: Inactivation of *L. monocytogenes* and *Salmonella* spp. after fermentation/drying ranged from ca. 1.1 to 1.3 and ca. 3.6 to 4.6 log CFU/g, respectively. Following HPP, numbers of *Salmonella* spp. were reduced by an additional 2.0 to 3.0 log CFU/g, whereas an additional decrease of 1.5 and 5.0 log CFU/g was achieved after 5 min at 70,000 psi and 3 min at 87,000 psi, respectively, for *L. monocytogenes*. After 28 days *L. monocytogenes* levels decreased by up to an additional 2.8 log CFU/g, whereas an additional decrease of up to ca. 0.5 log CFU/g was observed for *Salmonella* spp.

Significance: Fermentation/drying and/or HPP as a post-process intervention can appreciably reduce the levels of *L. monocytogenes* and *Salmonella* spp. in Genoa salami.

P1-23 DSC Growth of *Listeria monocytogenes* on Sliced Inoculated Pastrami and Roast Beef during Vacuum-packaged Storage at 4, 7 or 12°C

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Introduction: A recent outbreak of listeriosis in North America was traced to Ready-to-Eat meats produced by a single processing plant and resulted in the recall of potentially contaminated products including three brands of pastrami and ten brands of roast beef.

Purpose: This study examined the fate of *Listeria monocytogenes* on inoculated pastrami and roast beef to obtain information which may be useful in development/re-evaluation of pathogen risk assessments in Ready-to-Eat meats.

Methods: Commercially manufactured pastrami and roast beef (without or with 1.44% sodium lactate + 0.1% sodium diacetate; SL/SD) were sliced (0.2 cm thick; 5×5 cm), inoculated (1 to 2 log CFU/cm^2) with a 10-strain mixture of *L. monocytogenes*, vacuum-packaged, and stored at 4, 7 and 12°C for up to 90 d (two replications, three samples/treatment/sampling time/replication). Samples were analyzed periodically for pathogen (PALCAM agar) and total microbial (Tryptic Soy Agar with 0.6% yeast extract) counts. Microbial counts were fitted using DMFit software (equation of Baranyi and Roberts).

Results: In both pastrami and roast beef, lag phases of *L. monocytogenes* were extended and growth rates were reduced by SL/SD and lower storage temperatures. At all storage temperatures, pathogen growth rates on pastrami and roast beef without SL/SD were similar. However, in products with SL/SD and stored at 4 and 7°C, growth rates on pastrami were higher than those on roast beef. The longest lag phase (≥ 16 days) and the slowest growth rates (0–0.04 log CFU/cm^2/d) were observed on roast beef formulated with SL/SD and stored at 4°C. The fastest growth rate, 0.84–1.09 log CFU/cm^2/d,

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was observed on roast beef without SL/SD and stored at 12°C. Overall, the fate of total microbial populations was similar to that of *L. monocytogenes*.

Significance: These data should be useful in reduction of uncertainties in predictive models used in developing or updating quantitative risk assessments of *L. monocytogenes* in Ready-to-Eat meats.

P1-24 Microwell Format Detection Method for *Campylobacter* spp. in Foods Using DNA Hybridization

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Introduction: A microwell format DNA hybridization assay has been developed that employs DNA probes specific to *Campylobacter* spp. ribosomal RNA sequences in a solution phase hybridization, with detection by means of an enzymatically generated colorimetric endpoint.

Purpose: The GeneQuence *Campylobacter* assay is a DNA hybridization method for the detection of *Campylobacter* spp. in food samples. The assay has been validated for whole chicken carcasses, and application of the assay to other sample types is currently being developed.

Methods: For the currently validated method, *Campylobacter* recovery was achieved by chicken carcass rinse in 200 ml buffered peptone water. A one milliliter sample was taken from the rinse and enriched for 4 h at 37°C in supplemented Hunt broth under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2). Cefoperazone was then added for *Campylobacter* selectivity and incubation was continued at 42°C for 20 h. Campy-Cefex agar plates were then swabbed with the broth and incubated at 42°C for 24 h. Growth was collected from the Campy-Cefex plate into one milliliter buffered peptone water for analysis. Enrichments were treated with lysis reagent and rRNA targets were captured by means of DNA sandwich hybridization in microwell plates. Hybridization required one hour at room temperature and because of the uniqueness of the target sequence, it required no additional specificity control. Following hybridization, the plates were washed and enzyme substrate was added. The presence of *Campylobacter* spp. was indicated by enzymatic activity resulting in the development of blue color. Sulphuric acid was added to stop the reaction. Results were then obtained by measuring absorbance at 450 nm.

Results: Sensitivity of 1000 CFU per chicken carcass has been demonstrated for the assay by analysis of spiked rinses, with inoculation levels determined by MPN. The method may be adapted for lower tolerance limits by adjusting the amount of rinse sampled for enrichment. The assay has been shown to be fully inclusive for *C. coli* (13 strains), *C. jejuni* (34 strains), and *C. lari* (5 strains), and fully exclusive for closely related non-*Campylobacter* species and other potentially encountered poultry microflora (30 strains across 14 genera).

Significance: The development of this assay is applicable to food industry testing of commodities for contamination by *Campylobacter* spp.

P1-25 *Campylobacter jejuni* Detection in Chicken Grow-out Houses by Environmental Sampling Methods
DSC

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Introduction: Campylobacter and *Salmonella* are foodborne pathogens commonly associated with raw poultry. Although there has been much research done on isolating these pathogens from the poultry production environment using cloacal swabs, fecal samples, and intestinal tract dissection, there has been less research involving environmental sampling. The use of PCR to analyze environmental samples would minimize the need for organism specific media while also detecting any *Campylobacter* or *Salmonella* that may be injured.

Purpose: Improved environmental sampling methods provide an easy, convenient, and less time-consuming way to sample the poultry house environment for pathogenic organisms. Coupling this with PCR analysis can provide a relatively simple, rapid, and accurate way to test for these pathogens in a chicken house or flock prior to slaughter.

Methods: Air, boot, and sponge samples were collected from three commercial chicken grow-out houses when flocks were three, four, and five weeks old. Air samples were collected onto gelatin filters. Fecal/litter samples were collected from disposable booties. Pre-moistened sponges were used to sample feedpans and drink lines. A PCR method was used to qualitatively detect *Campylobacter jejuni* and *Salmonella* spp., simultaneously, in each sample.

Results: Campylobacter jejuni positive samples were found at each farm (house), at age three, four, and five weeks, and from each sample type. For all 270 samples, 110 (41%) were positive for *Campylobacter* and 0% for *Salmonella*. Collectively, 28% of air, 44% of sponge, and 50% of bootie samples were positive for *Campylobacter*.

Significance: Environmental sampling as a pre-slaughter test for foodborne pathogens attributed to poultry deserves further investigations. The methods used in this study are non-invasive to live animals, relatively rapid and specific, and could enable poultry processing facilities to coordinate scheduled processing of flocks with lower pathogen incidence, as a way to reduce post-slaughter pathogen transmission.

P1-26 Occurrence of *Campylobacter* spp. in Beef Carcasses and in Retail Chicken Cuts in São Paulo, Brazil

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Introduction: Campylobacter spp. is a relevant foodborne bacterial pathogen linked to undercooked meat consumption. Slaughtering operations have been determined as the main source of meat contamination by thermophilic *Campylobacter* species. In addition, the pathogen has been found to survive during chilled storage of meats.

Purpose: The aim of this study was to evaluate the prevalence and population of thermophilic *Campylobacter* species (*C. jejuni*, *C. coli* and *C. lari*) in beef carcasses and chicken cuts obtained from a Brazilian slaughterhouse and retail, respectively.

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Methods: 198 bovines (hides and respective carcasses) were sampled using swab (400 cm^2) in the breast region before and after dehiding. 85 different chicken cuts were sampled using rinsing methods. ISO 10272-1 method was used for detection and quantification of *Campylobacter* spp. and multiplex PCR to identify the isolates at genus and species levels.

Results: C. jejuni was found only on the hide of 45 (11.4%) of the samples from the 198 animals analyzed. No *Campylobacter* was found in the beef carcasses. For the chicken cuts, *C. jejuni* was isolated on 14 (16.5%) out of the 85 samples. *C. coli* and *C. lari* were not found either in bovine or chicken samples. *Campylobacter* population levels in chicken samples were < 2 CFU/g and < 0.01 CFU/cm^2 in bovine carcasses.

Significance: Data from bovine carcass samples indicate that the transfer of *C. jejuni* from hides to meat surfaces is low if good hygienic practices are used. Data on chicken cuts highlight the need for better hygienic controls from slaughter to retail markets. This study has shown that *C. jejuni* is the most prevalent species in both Brazilian bovine carcasses and chicken cuts. These data will be combined with those from other foodborne pathogens in a risk assessment model.
Acknowledgment: FAPESP

P1-27 Cytotoxic Potential of *Campylobacter jejuni* Isolated from Retail Poultry Samples

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Introduction: Approximately 2.1 to 2.4 million cases of campylobacteriosis are recorded in United States each year. Case-control studies in United States have shown that 48% to 70% of all sporadic infections are due to consumption of poultry contaminated with *Campylobacter jejuni*. According to Centers for Disease Control and Prevention (CDC), 80% of all retail chickens are contaminated with *Campylobacter jejuni*.

Purpose: The objective of this study was to investigate the pathogenicity of *Campylobacter jejuni* strains isolated from retail poultry samples on Chinese Hamster Ovarian (CHO) tissue culture cell lines.

Methods: Thirty-four *Campylobacter jejuni* strains isolated from retail poultry samples were analyzed using Bolton broth as pre-enrichment broth and modified Charcoal-Cefoperazone Deoxycholate agar (CCDA) as a selective media. The isolates were characterized the presence of 16 S-RNA gene PCR; the ability to invade and adhere to CHO Tissue Cells; and cytotoxic potential, using an in vitro lactate dehydrogenase (LDH) release assay as an indicator of cytotoxicity.

Results: Of the thirty-four strains isolated, 13 (38.2%) were positive for the presence of 16s RNA gene. The release of lactate dehydrogenase from Chinese Hamster cells resulted in cytotoxicity percentage of 99% for *C. jejuni* ATCC 29528, compared to study isolates, which exhibited a lactate dehydrogenase release ranging from 28% to 180%.

Significance: Thus, this study indicates that the highly pathogenic strains of *C. jejuni* can be isolated from retail poultry products, which can pose a threat to consumers.

P1-28 Biogenic Amine Production in Yellowfin Tuna (*Thunnus albacares*) under Controlled Decomposition Conditions

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Introduction: Scombrotoxin poisoning is a leading cause of illness associated with consumption of tuna. Biogenic amines (produced by spoilage bacteria), primarily histamine, accumulate in edible muscle tissue when tuna are time-temperature abused and may result in illness when consumed at elevated concentrations. Effective time-temperature controls are necessary to minimize the food safety risk associated with tuna consumption.

Purpose: This study investigated the time-temperature conditions of handling and iced storage of large tuna (eviscerated vs. uneviscerated) that lead to production of histamine at concentrations indicative of decomposition (50 ppm) and/or potential toxicity (500 ppm), as well as production of other biogenic amines. Cooling curve data were generated to better understand chill rates of eviscerated and uneviscerated tuna.

Methods: Live tuna were harvested, pithed, fitted with temperature data loggers, incubated in seawater at 30°C for 0 to 15 h, and then stored in ice in an eviscerated or uneviscerated state. Chilled fish were subsequently cut into sections, vacuum packaged, and frozen for transport and storage prior to analyses. Histamine analyses were conducted according to AOAC Official Method 977.13 and putrescine and cadaverine were analyzed by AOAC Official Method 996.07. Sensory analyses were performed by a cadre of trained seafood sensory analysts.

Results: Biogenic amine concentrations generally increased in large tuna with temperature and time of incubation in seawater. Eviscerated (n = 5) and uneviscerated tuna (n = 8) cooled to an internal temperature of 10°C in a mean of 11 h (range 9 to 13 h) and 14 h (range 9 to 21 h), respectively. Eviscerated tuna had maximum histamine (3,075 ppm) and cadaverine (101 ppm) concentrations after 15 h incubation in seawater at 30°C, whereas uneviscerated tuna had maximum histamine (5,525 ppm) and cadaverine (45 ppm) concentrations after 15 and 12 h incubation in seawater at 30°C, respectively.

Significance: The results generated in this study can be applied by the FDA to further develop guidance and policy for industry, leading to improvements in the harvesting and handling of large tuna that could result in a safer seafood product for consumers.

P1-29 Levels of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* in Intestinal Contents of Fish from the United States Gulf Coast

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Introduction: Vibrio spp. cause seafood-associated illnesses globally. Finfish are potential reservoirs and are important vehicles of *Vibrio*-associated illnesses in Asia, where they are often consumed raw. Limited quantitative data are available on *Vibrio* levels in finfish intestinal contents.

Purpose: This study examined the effect of feeding habits and environment on the abundance of pathogenic vibrios in the intestinal contents of common finfish species in the Gulf of Mexico.

Methods: Ten common finfish species from inshore (Mobile Bay) and offshore (Gulf of Mexico) were collected in fall of 2008. Their intestinal contents were examined by a variety of molecular methods in a most probable number (MPN) format to determine levels of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*. The Qualicon Bax Q7 real-time PCR system was used to screen overnight alkaline peptone water enrichments for genes specific to each of the *Vibrio* spp. These enrichments were further examined for a variety of pathogenicity markers, using real-time PCR and loop mediated amplification assays.

Results: Levels of *V. parahaemolyticus* and *V. vulnificus* often exceeded 10,000,000 MPN/g and appeared to be influenced by both feeding habits and environment. Highest levels were found in bottom dwelling fish from Mobile Bay that typically feed on invertebrates. Piscivores (mackerel) collected offshore harbored few vibrios (< 3 MPN/g). Salinity appeared to be influential in the distribution of these vibrios, with *V. cholerae* detection (< 10 MPN/g) being limited to low salinities (< 15ppt). *V. vulnificus* was prevalent at all inshore sites. *V. parahaemolyticus* was most widely distributed and more abundant than other *Vibrio* spp. in fish collected offshore in the Gulf (maximum 9300 MPN/g).

Significance: These data indicate that extremely high levels of vibrios can occur in the intestines of finfish, depending on harvest environment and feeding habits. Preventing contamination of edible flesh during evisceration and filleting may greatly reduce risk.

P1-30 DSC Evaluation of Immunomagnetic Separation (IMS) Coupled with Real-time PCR for Enumeration of *Vibrio parahaemolyticus* in Spiked Oyster Homogenates

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Introduction: Vibrio parahaemolyticus is a naturally occurring marine bacterium that causes a majority of the seafood-associated gastroenteritis cases in the United States. There is a need for an approved and standardized method that is sensitive, reliable and capable of accurate identification of *V. parahaemolyticus*.

Purpose: The purpose of this study is to develop an antibody based method coupled with real-time PCR (q-PCR) for enumeration of *V. parahaemolyticus* in spiked oyster homogenates.

Methods: The monoclonal antibody (MnAb) produced against the flagellar cores of *V. parahaemolyticus* was used in an immuno-magnetic separation (IMS) protocol. Live oysters were shucked, weighed and mixed in a ratio of 1:1(wt/wt) with sterile PBS and stomached for 2 min. Oyster homogenate was filtered and spiked with a serially diluted overnight grown culture of *V. parahaemolyticus* ATCC 33847. One ml of spiked homogenate dilution was subjected to the IMS protocol. The magnetic beads were collected, using a magnetic concentrator and the supernatant fluid was spread on VVA agar plates. For q-PCR, the magnetic beads were collected from the selected dilution, washed three times with PBS and suspended in 100 µl sterile water. The sample was then heated at 100°C for 15 min, and centrifuged at 16000 times g for 10 min; the supernatant was used as the DNA template in q-PCR.

Results: An average binding of 69% was exhibited in the binding study using the IMS protocol. When IMS was coupled with q-PCR, after successive washing steps, the binding capacity decreased to 40% after the first wash, 17 % after the second and 10% after the third.

Significance: IMS coupled with q-PCR method could be used to concentrate and detect *V. parahaemolyticus* from mixed cultures, seawater and shellfish homogenates.

P1-31 Validation of a PCR Assay for Screening *Vibrio* in Foods

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Introduction: The species of *Vibrio* that cause the majority of human illness are *cholera* (Vc), *parahaemolyticus* (Vp), and *vulnificus* (Vv). Since culture-based screening methods can be difficult and time-consuming, well validated, rapid methods for the detection of these pathogens are needed.

Purpose: This study evaluated the inclusivity, exclusivity, and effectiveness of a real-time multiplex PCR assay for detecting *Vibrio*.

Methods: Inclusivity testing (n = 50 strains) was performed at ~10^5 CFU/ml, while exclusivity testing (n = 50 strains) was performed at ~10^8 CFU/ml from broth cultures. For spiked foods, *Vibrio* strains were inoculated to yield fractional positive results for plus/minus screening, or at levels informative of method performance for MPN-based approaches. Samples were tested with the FDA-BAM culture-based method and by PCR using the BAX® system. Ahi tuna was spiked at three levels with Vc and tested for presence or absence of target in sets of twenty 25 g sub-samples with five unspiked sub-samples, with PCR testing from the BAM enrichments. Similarly, five replicates of scallops were tested, using the MPN approach of the BAM spiked with Vv at a level giving fractional results for the fifteen 1 g samples, and each MPN tube was tested by PCR, as were five 25 g samples enriched in a comparable manner. Naturally occurring fractionally positive, low-level Vc in raw shrimp was also tested, using twenty 25 g samples with both the BAM method and PCR testing from the same enrichments.

Results: All inclusivity/exclusivity testing demonstrated expected results. For effectiveness testing, no statistically significant differences were found when comparing test and reference methods. In fact, comparing PCR and culture, results for the spiked ahi tuna (36 positive of 65 samples tested) and shrimp (5 positive of 20 samples tested) were identical, with no false negative or false positive results by PCR. Scallop data gave identical MPN results for test and reference methods, and 25g enrichments were all positive by PCR.

Significance: This data indicates that this PCR method for the detection of *Vibrio* is as effective as culture-based methods while providing significant time and labor savings.

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P1-32 Comparison of Molecular Detection Methods for *Vibrio* spp. in Oysters

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Introduction: Vibrios are a global concern for seafood safety, and many methods, both culture-based and molecular, have been introduced for their detection and enumeration in recent years. However, few studies have compared the performance of these methods.

Purpose: This study compares various molecular methods for detection of natural populations of total and pathogenic *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* in oysters subjected to various levels of temperature abuse. Comparison of these methods was assisted by utilizing personnel that were involved in the development and routine use of the various methods as sample analysts for their method.

Methods: This study employed the Qualicon BAX Q7 real-time PCR system for screening MPN enrichments for the presence of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*. Multiplex real-time PCR detection of total and tdh+ *V. parahaemolyticus* was conducted on the Cepheid SmartCycler II and compared to results for total and tdh+ *V. parahaemolyticus* detected by LAMP assays. *V. vulnificus* confirmation was performed using two different real-time PCR methods, on the SmartCycler II and the ABI 7500.

Results: The three methods for detection of *V. paraehamolyticus* (BAX, SmartCycler, and LAMP) and *V. vulnificus* (BAX, SmartCycler, and ABI) were in agreement for 100% and 97% of the tests (n = 138), respectively. The multiplex real-time PCR SmartCycler method was in 71% agreement with the LAMP assay for detection of tdh+ *V. parahaemolyticus* (n=129); however, when the same tdh assay was run alone (not in multiplex), there was 98% agreement with the LAMP results.

Significance: The results of this study indicate the benefit and limitations of some of the currently available molecular methods for detection of vibrios. This improved understanding of assay performance will allow better interpretation of results obtained with these methods, leading to a more accurate assessment of the public health risk when these pathogens are detected.

P1-33 Heat Inactivation of Enteric Viruses in Soft Shell Clams

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Introduction: Hepatitis A virus (HAV) and norovirus (NoV) are among the most incriminated agents of foodborne diseases. Among the identified vehicles, bivalve molluscs are the most incriminated in the transmission of viruses. Very little work has been reported on the efficacy of heat treatments for the inactivation of enteric viruses in molluscs and especially in soft shell clams (*Mya arenaria*).

Purpose: The objective of this study was to establish a heat treatment procedure for the inactivation of enteric viruses in soft shell clams.

Methods: Spiking of shellfish samples was performed in digestive glands with HAV or murine NoV. Different heat treatments were applied in both glass jar and plastic bags in the presence of mollusc meat, using different internal temperatures and for different periods of time.

Results: Results have shown that complete inactivation (5 log reduction) was achieved at 90°C for 180 s and for 300 s for murine NoV and HAV, respectively, when the digestive gland was alone in the glass jar. In plastic bags, when the spiked gland was in the presence of mollusc meat, a treatment of 300 s at 90°C was also necessary to ensure a complete reduction of HAV.

Significance: Our results have shown that HAV is more resistant than murine NoV and that relatively severe heat treatments are required to ensure complete viral inactivation in soft shell clams. These results may be used as a basis for establishing efficient strategies to control enteric viruses and assure safety in bivalve molluscs.

P1-34 Minimum Safe Cooking Temperatures for Eliminating Foodborne Pathogens in Shrimp
DSC

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Introduction: Shrimp, one of the most common seafoods, is a favorite of consumers. As with many other foods, there are safety concerns about shrimp. *Listeria* spp., *Salmonella* spp., *Clostridium* spp. and *Vibrio* spp. are among the pathogens of prime importance. Most of these pathogens can be eliminated by cooking. However, the extent and temperature of cooking can greatly influence the safety of seafood.

Purpose: The current study is focused on the determination of minimum cooking temperatures for reducing *Listeria* spp., *Salmonella* spp. and *Vibrio* spp. to non-detectable levels on the surface of shrimp.

Methods: Shrimp were surface inoculated with the three different species mentioned above to about 5 log CFU/g of shrimp and then incubated for two days. Shrimp samples were treated at five different temperatures on 0, 1 and 2 day by boiling in a water bath. The effects of temperature on bacterial counts were determined by plating and calculating the log CFU/g reduction for each temperature. The experiment was repeated with different temperatures for each bacterium until the bacterial load in the shrimp was at non-detectable levels.

Results: An internal temperature of 85°C was the lowest temperature needed to kill all the bacteria tested. *Vibrio* spp. was the least resistant to heat, with bacterial counts reaching non-detectable levels at 55°C. For *Salmonella* spp. the minimum temperature required to reduce bacterial counts to non-detectable levels was 75°C, while *Listeria* spp. showed the highest resistance, up to 85°C.

Significance: This study is mainly intended to design a simple, easy and unbiased consumer guide for cooking shrimp to enhance safety of shrimp handled and cooked at home. It can also serve as a guide for manufacturers of Ready-to-Eat shrimp products in designing and planning GMPs and HACCP plans for production.

P1-35 Effect of Gamma Irradiation on Inactivation of Foodborne Virus in Oyster

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Introduction: Norovirus is well known as a pathogen in water and foods, but a study of norovirus in shellfish is complicated by a lack of enumeration methods for the virus.

Purpose: Therefore, the objective of this study was to evaluate the inactivation effect of gamma irradiation on foodborne virus in oyster, using poliovirus as a surrogate for norovirus.

Methods: In order to evaluate the antimicrobial effect of irradiation, 0.1 ml (6.5 log PFU/ml) of poliovirus (serotype poliovirus-1, ATCC VR-1562) was inoculated into 100 ml of phosphate buffered saline (PBS), and the samples were then gamma-irradiated at 0, 0.1, 0.2, 0.3, and 0.5 Gy. In addition, 40 oysters (*Crassostrea gigas*) within shells were placed in 7 L of artificial seawater (3% sea salt; wt/vol) contaminated with 4.9 log PFU/ml of poliovirus at 16°C in the dark for 2 h. Shells of oysters were then removed and the flesh submitted to gamma irradiation at 0, 0.5, 1, 2, 3, 4, and 5 kGy. Populations of the virus was enumerated by TCID50 (tissue culture infectious dose, log), and plaque forming unit (log) of poliovirus was further used to calculate D10 values by simple regression, and D_{12} values were also determined.

Results: The number of poliovirus in PBS decreased ($P < 0.05$) by 2.7 log PFU/ml after irradiation, and the initial virus populations (5.4 log PFU/oyster) in oyster samples decreased ($P < 0.05$) significantly to 3.5 log PFU/oyster as irradiation dose increased. Moreover, D10 values in PBS and oysters were 0.46 kGy and 2.84 kGy, respectively, and D12 values were 5.52 kGy and 34.08 kGy for PBS and oyster, respectively.

Significance: These results suggest that gamma irradiation may be useful in destroying foodborne virus in oysters, and food substrates may influence the antiviral effect of irradiation on foodborne viruses.

P1-36 Norovirus Detection and Quantification in Shellfish Samples

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Introduction: Noroviruses (NoVs) are the major agent responsible for nonbacterial gastroenteritis in humans and are present in large quantities in sewage during epidemic season. Shellfish concentrate NoVs in their digestive tissues when exposed to waters contaminated with sewage. As a consequence, NoVs are the most common cause of gastroenteritis outbreaks linked to shellfish consumption.

Purpose: The finding that most NoVs are concentrated in digestive tissues led a decade ago to the development of a specific, reliable and reproducible method based on virus elution and concentration by PEG precipitation.

Methods: Nucleic acid extraction and purification is accomplished with a kit based on magnetic silica, and viral RNA is detected in a one-step real time RT-PCR assay. By use of this method, six samples can be analyzed in one working day. An extraction control virus (Mengo virus) included in the assay, as well as internal controls, are employed as controls of virus and nucleic acid extraction and RT-PCR, respectively.

Results: We evaluated the performance of this assay in bioaccumulated and naturally contaminated oysters. We observed good virus recovery and assay reproducibility for GI and GII NoVs and Mengo virus in bio-accumulated oysters. Triplicate extractions showed less than 0.5 log variations. Similar reproducibility was observed in the detection of GII NoVs in naturally contaminated oysters, but greater variability was seen for GI NoVs. The inclusion of control RNA and Mengo virus excluded the presence RT-PCR inhibitors as a possible explanation for the observed differences. Increased variability of the distribution of GI NoVs within oyster beds is a possible explanation for this result. This method was applied to estimate the level of NoV contamination in naturally contaminated shellfish (n = 100). The geometric mean concentrations of GI and GII NoVs, corrected for extraction efficiency, were 1,300 and 525 RNA copies/g of DT, respectively. This method was also applied to bioaccumulated oysters that were then subjected to cooking.

Significance: In summary, this method for detection of NoV in shellfish is quite easy to perform and allows the analysis of several shellfish at one time. The rapidity of the assay may allow producers to evaluate their product prior to marketing. This quantitative approach also can be used for risk analysis, evaluation of virus persistence within the oyster, assessment of the impact of sewage in contaminated areas, and determination of the effectiveness of depuration.

P1-37 Characterization of *Salmonella* spp. from Nopal Leaves and Associated Soil and Water Samples in Morelos, Mexico

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Introduction: Introduction: Nopal is a native cactus species [*Opuntia ficus-indica* (L.) MILL (Cactaceae)] of great economic importance in Mexico. It is grown in open fields and subsequently ingested fresh as a salad or processed as a juice or yogurt, but it may also be used as a dietary supplement and/or for cosmetic products. Recent *Salmonella* outbreaks have resulted in renewed concerns about the incidence and control of this bacterium, particularly in fresh fruits and vegetables such as cantaloupe, tomatoes, bell peppers, and jalapeños.

Purpose: The objective of this study was to determine the bacteriological safety of fresh cactus leaves (nopal) and the associated soil and water in Morelos, a cactus-producing region of south central Mexico.

Methods: A total of 34 samples (18 cactus leaves, 8 soil, and 8 water) were taken randomly from the high, middle, and low levels of a nopal production field between May and June of 2006. Traditional microbiological methods (NOM-114-SSA1-1994) and PCR were used to determine the prevalence of the pathogen and serotyping, ribotyping, and PFGE were used to further confirm and characterize the isolates.

Results: Based on both microbiological and PCR analyses, 23 of 34 total samples (67.4% prevalence; 12 positive samples from nopal leaves, 7 from surrounding soil, and 4 from pond/irrigation water) were positive for *Salmonella* spp. Serotyping revealed that the isolates were either *S.* Typhimirium or *S. javiana*. The results from PFGE of selected isolates (10 from cactus leaves, 3 from soil, and 2 from water) revealed that the isolates displayed 3 pulsotypes. Most isolates (8 of 16 isolates; 2 from water and 6 from nopal leaves) belonged to pulsotype III, whereas pulsotype I contained 6 isolates (4 from nopal leaves and 2 from soil) and pulsotype II contained 2 isolates (both from soil). It was possible to associate strains from nopal leaves with those recovered from water or soil based on pulsotype, but it was not possible to associate isolates from soil with those from water based on PFGE.

Significance: These data suggest that nopal may become contaminated with *Salmonella* from both water and soil and suggests that it may serve as a vehicle for transmission of this pathogen to the public, particularly those at higher risk, who may consume fresh or processed nopal.

P1-38 Survival of *Salmonella* spp. during Preparation of Pancakes and Waffles

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Introduction: Salmonella contamination has been the cause of several food product recalls, including dry pancake and waffle mixes.

Purpose: The purpose of this study was to evaluate the survival of *Salmonella* spp. during pancake and waffle preparation.

Methods: The pancake and waffle batters were prepared from two types of mix, original and buttermilk, as per manufacturer's instructions by adding milk or water, oil and egg. The batter was mixed uniformly to minimize the lumps. The batter was inoculated with a five strain cocktail of *Salmonella* spp. to obtain ca. 10^7 CFU/g of the batter. The pancakes were prepared on an adjustable temperature flat griddle, using different time-temperature combinations (250°F for 1.45, 2.00, and 2.15 min; 375°F for 1.15, 1.30 and 1.45 min; 425°F for 0.45, 1.00, and 1.15 min) , while waffles were made in a Belgium style waffle maker using three different settings (3, 4.5 and 6). The surviving *Salmonella* spp. populations were enumerated on XLD and PCA. Samples with *Salmonella* spp. populations below the detection limit were subjected to a modified FDA-BAM enrichment procedure.

Results: Salmonella spp. populations were reduced to below the minimum detection limit (0.699 CFU/g) when plated on XLD agar during preparation of the pancakes and waffles. *Salmonella* spp. populations were reduced by 4.6 to 5.6 and 4.3 to 6.0 log CFU/g in pancakes prepared from original mix and buttermilk mix, respectively. The microbial reductions were 6.2 to 6.9 log CFU/g in original mix waffles, and 5.4 to 6.1 log CFU/g in buttermilk mix waffles. *Salmonella* spp. was detected in almost all samples of pancakes and waffles prepared using various time-temperature combinations.

Significance: The pancake and waffle preparation methods were effective in reducing the *Salmonella* spp. populations. However, *Salmonella* spp. cells may survive the cooking process and pose a health risk for at-risk individuals.

P1-39 Survival of *Salmonella* spp. during Preparation of Popcorn

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Introduction: Salmonellosis ranks first among the foodborne outbreaks in the United States. The presence of *Salmonella* on corn has been reported in literature.

Purpose: We evaluated the survival of *Salmonella* spp. during popcorn preparation, using three different popping methods.

Methods: The corn obtained from a local grocery store was inoculated with a five strain cocktail of *Salmonella* spp. to obtain 6.84, 2.70, 0.99 log CFU/kernel. The control as well as inoculated corn were popped using three different methods: microwave, hot air and electric heater following manufacturers' directions. The surface temperature obtained during the three popping methods was measured using infra red imaging system. The surviving *Salmonella* on the popcorn was enumerated on selective xylose lysine desoxycholate (XLD) agar and non-selective Tryptic Soy Agar (TSA). For samples with *Salmonella* spp. populations less than the detection limit, its presence was detected using enrichment method outlined in bacteriological analytical manual (BAM).

Results: The maximum surface temperatures obtained during microwave, hot air and electric popping methods were in the range of 120° to 130°, 200° to 210°, and 230° to 240° C, respectively. *Salmonella* spp. was not detected on popcorn prepared from non-inoculated corn using the three popping methods. *Salmonella* spp. counts of popcorn prepared from inoculated kernels were below the detection limit (-0.81 log CFU/popcorn) on XLD agar. The corn inoculated to 6.84, 2.70, 0.99 log CFU/kernel and popped in microwave oven resulted in survival of 0.68, 0.55, <- 0.81 log CFU/popcorn, respectively on TSA. The corresponding values for popcorn prepared using electric and hot air popping methods were 0.44, 0.18, 0.06 and 0.34, 0.12, <- 0.81 log CFU/popcorn, respectively. *Salmonella* spp. was able to survive the popcorn preparation process using any of the three methods.

Significance: Salmonella spp. present on corn may survive the commonly used preparation methods used at home and proper care must be taken to prevent the contamination of the corn to minimize the risk.

P1-40 Alternative Cooking Procedures for Large, Intact Meat Products to Achieve Lethality Microbiological Performance Standards

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Introduction: Achieving FSIS lethality microbiological performance standards for cooking procedures proves to be challenging for large whole-muscle meat products. Failure to satisfy recommended time and temperature limits of a cooking process results in a deviation from a critical limit and requires corrective actions to be performed on all products associated with the deviation. Increasing acceptable cooking times will reduce the incidence of deviations and the false assumption of unsafe products.

Purpose: This study was conducted to determine if alternative heating times and temperatures other than those defined in Appendix A could be utilized and still comply with FSIS microbiological performance standards.

Methods: Large (10.43 to 12.25 kg) cured bone-in hams (n = 80) and large (≥ 9.07 kg), uncured beef inside rounds (n = 80) were utilized. The effect of alternative lethality parameters on toxin production of *Staphylococcus aureus* and log reduction of *Salmonella* Typhimurium and coliforms was studied. Both the ham and roast beef were subjected to 1 of 10 treatments defined by varying final internal product temperatures (48.9°C, 54.5°C, 60.0°C, 65.6°C, or 71.1°C) and relative humidities (50 or 90%).

Results: Results of the study showed at least a 6.5 log reduction in *S.* Typhimurium across all lethality treatments for both products. Further, coliform counts also were significantly reduced, and *S. aureus* toxin kits returned negative results for toxin production across all treatments for both ham and roast beef. In addition, relative humidity did not alter lethality effectiveness.

Significance: This study supports product safety with the use of heating times and humidities other than those specified by Appendix A. The results demonstrate that industry may have increased flexibility associated with heating large, whole-muscle cuts while still complying with the required performance standards.

P1-41 Survival and Growth of *Salmonella* in Salsa and Related Ingredients

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Introduction: A large outbreak of salmonellosis associated with jalapeño peppers, serrano peppers, and possibly tomatoes occurred in the United States in 2008. During the outbreak, two clusters of cases were identified as being significantly associated with eating salsa.

Purpose: To determine the survival and growth characteristics of *Salmonella* in different salsa preparations and related principal ingredients: tomatoes, jalapeno peppers, and cilantro.

Methods: Intact and chopped vegetables and different formulation salsas were inoculated with a five-strain mixture of *Salmonella* spp. including *S.* Typhimurium and *S.* Enteritidis and stored at 4, 12, and 21°C for up to 7 days. *Salmonella* populations and total aerobic counts were monitored during storage.

Results: Salmonella did not grow but survived on intact tomatoes and jalapeno peppers (initial inoculation ca. 6 log CFU per tomato or jalapeno pepper), whereas significant growth of *Salmonella* occurred on the surface of intact cilantro at 12 and 21°C, increasing from 3.48 log CFU/g to 5.39 log CFU/g at day 7 at 12°C and to 6.26 log CFU/g at day 5 at 21°C. In general, growth of *Salmonella* occurred in all chopped vegetables when stored at 12 and 21°C, with chopped jalapeno peppers being the most supportive of the growth. Regardless of the differences in salsa formulation, no growth of *Salmonella* (initial inoculation ca. 3 log CFU/g) was observed in salsa held at 4°C; however, rapid or gradual decreases in *Salmonella* populations was only observed in formulations that contained both fresh garlic and lime juice. *Salmonella* grew in salsas at 12 and 21°C, except for salsa formulations that contained both fresh garlic and lime juice, in which the inoculated *Salmonella* decreased by ca. 3 log CFU/g within 3 to 7 days of storage.

Significance: The results highlight the importance of preharvest pathogen contamination of fresh produce, salsa formulation, and temperature control in preventing salmonellosis.

P1-42 DSC PCR-DGGE Analysis of Microbial Communities Associated with *Campylobacter* spp. on Meat Contact Surfaces in a Pork Processing Facility

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Introduction: Campylobacter spp. cause the greatest number of bacterial foodborne illnesses in the developed world and meat has been implicated as a vector for transmission. Previous research demonstrated that *Campylobacter* spp. do not survive on meat unless an associated microflora is present.

Purpose: The objectives of this research were to use molecular methods to investigate the diversity of the bacterial population in a meat processing plant and to determine the type of microflora associated with *Campylobacter* spp. on meat contact surfaces.

Methods: Swab samples from meat contact surfaces (stainless steel or silicon) were collected from a pork processing facility, suspended in peptone water and divided for analysis. A portion was enriched and plated for isolation and identification of *Campylobacter* spp. and a portion was plated onto a variety of selective media for enumeration of different bacterial species and DNA isolation. PCR-denaturing gradient gel electrophoresis (DGGE) was used for molecular characterization of the bacteria community or *Campylobacter* spp.

Results: Analysis of PCR-DGGE profiles of the total population recovered from selective media (Plate count agar, MRS, violet red bile glucose agar, *Pseudomonas* CFC selective agar) revealed that neither the type of surface material nor the

composition of the microbial community impacted the presence of *Campylobacter* spp. The PCR-DGGE analysis of the microbial communities obtained from selective media showed diverse microbial populations at each sampling site, with some particular microbial species present consistently at all locations from the kill floor to the cutting room. In addition, the type of surface material did not affect the composition of the microbial community.

Significance: The outcome of this research is critical for future research on the ecology of *Campylobacter* spp. in meat processing facilities. Knowledge of the microbial diversity associated with *Campylobacter* spp. that may allow it to survive on meats and in processing facilities will allow development of targeted interventions that could reduce the risk from this meatborne pathogen.

P1-43 Improved Enrichment of *Shigella* spp. in Produce

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Introduction: The US Food and Drug Administration (FDA) routinely monitors fresh produce for bacterial pathogens. Detection of *Shigella* spp. is particularly challenging, and modifications to current procedures are being evaluated.

Purpose: To determine whether a novel acid enrichment procedure can improve the isolation of *Shigella* spp. by minimizing growth of the high levels of epiphytic bacteria typical of fresh produce.

*Methods: S*pinach, parsley and green onion rinsates, containing approximately 10^6 CFU/ml epiphytic bacteria, were inoculated with *Shigella flexneri*, *S. dysenteriae*, *S. boydii* and *S. sonnei* at approximately 10 CFU/ml. The spiked rinsates were enriched both by the current FDA import produce procedure and an experimental acid enrichment. After 24 h, populations were enumerated by plating on Rainbow Agar *Shigella*, Hektoen Enteric Agar and Trypticase Soy Agar plus Yeast Extract. Quantitative real-time PCR was also utilized to estimate populations, using ipaH and mxiC genes. Pure cultures of 19 strains of *Shigella* spp. were also enriched by the standard and acid procedures, as well as with non-selective Tryptic Soy Broth (TSB), and subsequently enumerated.

Results: Target *Shigella* spp. populations were greater after acid enrichment of spiked rinsates in approximately 60% of samples. With pure cultures, acid enrichment yielded the largest population of *Shigella* spp. in 54 of 57 enrichment/plating combinations. In 19 of 19 trials, acid enrichment produced larger *Shigella* spp. populations than growth in nonselective Trypticase Soy Broth.

Significance: The current enrichment procedure appeared to produce large populations of cells, as indicated by real time PCR detection of *Shigella*-specific genes. However, target populations were commonly outnumbered by nontarget populations at ratios over 500:1, making detection extremely difficult even on selective agars. The acid enrichment procedure appeared to both yield more target cells and reduce competing microflora, thus improving the possibility of isolating *Shigella* spp. for subsequent biochemical differentiation and epidemiology.

P1-44 Resistance of *Listeria monocytogenes* to Gamma Irradiation in the Presence of Glucose and NaCl

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Introduction: Gamma irradiation has been shown to have an antimicrobial effect on *Listeria monocytogenes*, but influences of food substrates on irradiation efficiency have not been studied.

Purpose: The objective of this study was to evaluate the effect of glucose on *L. monocytogenes* survival under sequential stresses of gamma irradiation and NaCl.

Methods: One milliliter of *L. monocytogenes* (5-strain composite) was inoculated (7-8 log CFU/cm2) in 40 ml of phosphate buffered saline (PBS) plus glucose (0, 2, 4%). The samples were then gamma-irradiated at 0, 0.1, 0.2, 0.3, 0.4 and 0.5 kGy, and the samples were exposed to NaCl (0, 1.5, 3.0, 4.5, 6.0, 7.5, 9%) in Tryptic Soy Agar plus 0.6% yeast extract by plating. All plates were incubated at 30°C for 48 h to enumerate surviving cells of *L. monocytogenes* (two replications, two samples each). The cell counts were further used to calculate D10 values (the dose to reduce 1 log) using a simple regression, and t3D values (tshoulder+3D10) were also determined. Moreover, a model was developed to predict D10 values as a function of glucose and NaCl concentrations, using the polynomial equation.

Results: Cell counts of *L. monocytogenes* decreased ($P < 0.05$) as irradiation dose increased, and *L. monocytogenes* in PBS (no glucose) was more ($P < 0.05$) sensitive to irradiation and NaCl compared to those in 2 and 4% glucose. D10 values were 0.07–0.1 kGy, 0.12–0.16 kGy, and 0.13–0.15 kGy for 0%, 2%, and 4% glucose, respectively. The t3D values were 0.22–0.3 kGy (0%), 0.35–0.48 kGy (2%), and 0.4–0.44 kGy (4% glucose). In addition, the developed model had a normal probability and an acceptable coefficient of determination (R2 = 0.715).

Significance: These results suggest that appropriate doses of gamma irradiation to destroy *L. monocytogenes* in foods should be determined according to the concentrations of glucose and NaCl in foods, and a predictive model may be useful in determination of irradiation doses.

P1-45 Effect of Pulsed Light Treatment on Growth and Resistance Behavior of *Listeria innocua* and *Escherichia coli*

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Introduction: Pulsed Light treatment is able to inactivate contaminating microorganisms in clear liquid solutions and on food and food contact surfaces. The non-linear kinetics of Pulsed Light inactivation has recently been documented, but there is little understanding of resistance and growth behavior of microbial survivors of Pulsed Light treatments.

Purpose: The objective of this study was to examine the growth and survival behavior of *L. innocua* and *E. coli* cells after various exposures to Pulsed Light treatment.

Methods: L. innocua (environmental isolate) and *E. coli* ATCC 25922) were grown to stationary phase in Tryptic Soy Broth (TSB) and diluted 10-fold in Butterfield's Phosphate Buffer. One ml volumes were exposed to Pulsed Light doses (fluence) of up to 13.3 J/cm^2 for both organisms. Surviving isolates from 1.1 and 10.1 J/cm^2 were recovered and repeatedly exposed to additional Pulsed Light treatments. Untreated, single, and double Pulse Light exposed isolate survival curves and 24 h growth curves were generated and statistical differences were determined using two-way ANOVA.

Results: Growth curves of *L. innocua* and *E. coli* isolates that were not exposed to Pulsed Light were not significantly different from growth curves of isolates exposed to 1.1 or 10.1 J/cm^2 or isolates from a subsequent (second) exposure to Pulsed Light. The Pulsed Light inactivation curves for both unexposed organisms and isolates recovered from single or repeated exposure to Pulsed Light were not significantly different. Initial reductions after treatment with 1.1 J/cm^2 ranged from 2.6 to 3.8 log CFU/ml while a reduction of from 5.0 to 5.8 was reached after exposure to 13.3 J/cm^2.

Significance: The results of this study show that Pulsed Light treatments can significantly reduce *L. innocua* or *E. coli* in clear liquids. The surviving cells did not exhibit changes in resistance or growth kinetics, which is important for practical applications of this technology.

P1-46 PFGE as a Predictor of *Listeria monocytogenes* Biofilm Formation

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Introduction: Persistence and spread of *Listeria monocytogenes* in food manufacturing and retail environments is greatly aided by the ability of this pathogen to form biofilms. While numerous *Listeria* isolates from environmental surveys have been serotyped and subjected to various strain-specific molecular typing methods, relatively few studies have characterized such strains in terms of biofilm formation, which could lead to improved *Listeria* intervention strategies.

Purpose: In this study, the relationship between biofilm formation, serotype and pulsed-field gel electrophoresis (PFGE) type was determined in a set of 30 *L. monocytogenes* environmental isolates from one delicatessen.

Methods: All 30 *L. monocytogenes* delicatessen isolates were serotyped using a PCR-based method with five different primer sets and then subjected to PFGE typing using the PulseNet protocol with restriction enzymes AscI and ApaI. Biofilm formation was quantified using a standard microtiter plate assay with Modified Welshimer's Broth as the growth medium. After averaging the 9 optical density readings from triplicate wells for each of three replicates, each isolate was classified as a weak, medium or strong biofilm former.

Results: Overall, 16, 7, 6 and 1 *L. monocytogenes* isolates belonged to serotypes 1/2b(3b), 1/2a(3a), 4b(d,e) and 4a/c, respectively. A total of 12 PFGE types grouped into three distinct clusters. All 7 strong biofilm-forming strains belonged to serotype 1/2a(3a) and to two closely related PFGE types containing a unique high kbp doublet using ApaI that were only 60.9% similar compared to the remaining 23 isolates. These 7 strong biofilm formers were isolated from multiple locations over 9 months. However, 4 weak and 2 medium biofilm-forming strains also persisted for 10 months, thereby weakening previous correlations between biofilm formation and persistence.

Significance: These findings demonstrate that PFGE typing can be a predictor of *L. monocytogenes* biofilm formation. However, other factors in addition to biofilm formation are also clearly important in persistence.

P1-47 Effect of Various Factors on the Formation of Biofilms by Four Strains of *Listeria monocytogenes*

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Introduction: It has been suggested that biofilm formation may be influenced by environmental factors. However, little information addresses the effect of multiple factors on biofilm formation by different strains of *L. monocytogenes*.

Purpose: The purpose of this study is to investigate the ability of four different outbreak-related strains of *L. monocytogenes* to form biofilms as a function of pH, temperature, water activity, inoculum size, and nutrition level.

Methods: Four *L. monocytogenes* isolates (FSLJ1-129, FSLR2-499, FSLJ1-003, FSLJ2-685) were screened for biofilm formation, using the microtiter plate method. Growth conditions were fixed for pH (5, 7, 9), water activity (0.92, 0.96, 0.98), nutrition level (0.25, 0.5, 0.75% of Tryptic Soy Broth), inoculum size (2, 4, 6 log CFU/mL), and three incubation temperatures (4, 22, 40°C). Biofilms were evaluated after 48 h of growth, and treated with crystal violet, after which optical density was measured at 595 nm. Data were analyzed by performing an analysis of variance in a full factorial design.

Results: Factors (pH, temperature, water activity and inoculum size) and strain significantly affected biofilm formation ($P < 0.05$). The effect of the nutrition level on biofilm formation was not significantly different ($P > 0.05$) among the evaluated strains. Interactions between several factors were significant ($P < 0.05$) for biofilm formation. Additionally, morphological changes in biofilms were observed when combinations of factors were evaluated.

Significance: These data are the first to demonstrate that multiple factors differentially influence biofilm formation by *L. monocytogenes* isolates. The findings may provide insight into how the pathogen persists in some food processing environments.

P1-48 Characterization of *Listeria monocytogenes* Isolates of Imported Cheese Contributed to the National PulseNet Database by the FDA from 2001 to 2008

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Introduction: Imported foods must meet the same Food and Drug Administration (FDA) standards as domestic foods. Pursuant to regulatory activities, FDA conducts compliance surveillance on imported foods offered for entry into US commerce. The FDA compliance surveillance system helps to detain or refuse entry of foods that are found to be

contaminated with *Listeria monocytogenes* (LM). PFGE and serotyping analysis of *Listeria* isolates have the potential to link imported cheese to outbreak associated or sporadic cases in the general population or clinical isolates in the National Pulsenet database.

Purpose: To characterize PFGE patterns and serotypes of *Listeria* isolates of cheese imported from different countries and compare the relatedness of the isolates.

Methods: FDA laboratories use PFGE to subtype foodborme pathogens isolates in imported foods and submit molecular patterns to the National PulseNet Database. FDA import samples are commodities collected from shipments made by foreign firms into the US. Import samples are collected either by investigational means, by mail entry, by domestic import or by official means. The samples collected are entered in the FDA Field Accomplishments and Compliance Tracking System (FACTS), which is an Agency-wide computer based program.

Results: There were 830 *Listeria* isolates in FDA FACTS, and 301 were *Listeria* isolates from imported foods. Sixty-one of the *Listeria* isolates were fromcheese imported from the following countries: Mexico, Italy, Israel, Portugal, Colombia, Greece and Spain. All isolates from Israel have the same PFGE pattern (GX6A16.0519) and all isolates from Portugal and Greece have identical PFGE pattern (GX6A16.0238). About 70 percent of the isolates were serotyped, and 52.4% of serotype 1 and 47.6% of serotype 4. All isolates from Mexico were serotype 4, while isolates from Israel and Portugal were serotype 1 and all isolates from Italy except one were serotype 1.

Significance: This study shows genetic diversity of *Listeria monocytogenes* isolates from imported cheese products coming to the US from different countries. Routine characterization of PFGE patterns and serotyping of imported foods can serve as a discriminating tool to show a geographic niche of *Listeria* serotypes in imported cheese.

P1-49 Viability of *Listeria monocytogenes* in Biofilms Exposed to Sanitizers, Osmotic Stress and Bacteriocins

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Introduction: L. monocytogenes is a foodborne pathogen that can persist in food industries as a member of microbial biofilms, which constitute a source of contamination difficult to control.

Purpose: To evaluate the influence of stress conditions on biofilms of *L. monocytogenes*.

Methods: Experiments were done at 37°C for 24 h, under agitation, with stainless steel coupons clamped vertically to a circular support immersed in Brain Heart Infusion broth (BHI). The BHI was inoculated with 10^5 CFU/ml of *L. monocytogenes*, under the following conditions: a) BHI broth (control); b) BHI plus 2% sucrose; c) BHI plus 5% NaCl; d) BHI plus crude extract containing bacteriocin from *Lactobacillus sakei* 1 (6,400AU/ml) and e) BHI plus crude extract containing bacteriocin of *Leuconostoc mesenteroides* A11 (3,200 AU/ml). Experiments were also done to evaluate the efficacy of peroxyacetic acid (1:40, v/v) and sodium hypochlorite (140 ppm of chlorine) on pre-formed *L. monocytogenes* biofilms. For all experiments, quantification of adhered cells was done by plate counting on Tryptic Soy Agar plus 0.6% yeast extract. Viability was also studied by double staining the surface of the coupons with the fluorescent dyes CTC-DAPI and direct counting under fluorescence microscopy. Biofilm formation was considered with at least 10^3 CFU adhered/cm^2.

Results: No difference ($P < 0.005$) was found by plating method for *L. monocytogenes* cultured with sucrose or NaCl, compared to control (ca. 5 log CFU/cm^2 in any condition). However, staining with CTC-DAPI revealed a difference in viability of *L. monocytogenes* in control and with NaCl ($P < 0.001$). Plate counting results showed bacteriocins of *L. sakei* and *L. mesenteroides* reduced adhesion of listerial population by ca. 3.3 and 2.8 log CFU/cm^2, respectively ($P < 0.001$). Results of CTC-DAPI showed a reduction of ca. 1 log CFU/cm^2 of *L. monocytogenes*/cm^2 in the presence of both bacteriocins. After treatment of pre-formed biofilms with peroxyacetic acid, no viable cells were detected by plate counting and hypochlorite reduced 1.8 log CFU/cm^2. CTC-DAPI staining showed reduction of 3.0 and 3.5 log CFU of *L. monocytogenes*/cm^2 in pre-formed biofilms, respectively, after treatment with hypochlorite and peroxyacetic acid.

Significance: CTC-DAPI was important to accurately determine viability of *L. monocytogenes* in biofilms under stress conditions.

P1-50 Optimizing Sampling Plans for Identifying Sources of *Listeria monocytogenes*: An Example from a Multi-state Turkey Processing Plant Study

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Introduction: Processors increasingly rely on microbiological sampling of the plant environment to determine if their products or processes are at risk of containing or transmitting pathogens, including *Listeria monocytogenes* (*Lm*). Appropriate evaluation of the sampling plan and test results over extended times should lead to changes in the test sample quantity, type, frequency, time, location or analysis.

Purpose: Food processors need tools to develop and justify sampling plans that optimize identification of microbial sources or niches with a reasonable number of environmental samples.

Methods: Environmental and raw product samples from commercial turkey slaughter plants in five states were analyzed for *L. monocytogenes*. All samples were classified into six sample types (drains; walls/ floors; product contact surfaces and equipment; process/ chilling water; workers, gloves and boots; post-chill raw product) from one of four processing areas (transport through defeathering; evisceration; chilling; post-chill cut-up/ packaging).

Results: For the 1,587 samples collected, 305 (19.2%) were positive for *Listeria* spp., and 97 (6%) were positive for *Lm* More than one *Lm* serotype was identified in many samples. A processing plant schematic illustrates the location of these positive samples. The proportion of positive samples from drains was twice as high as for other sample types. The

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proportion of *L.m.* to *L. innocua* positive samples was > 2:1 from the chilling and post-chill cut-up areas, and < 1:2 from transport to defeathering and the evisceration area. Serotype 4b, which is responsible for most large listeriosis outbreaks in the US, was identified from each sample area and from all sample types except water and worker, gloves and boots.

Significance: This analysis can be used for other data sets to determine which combinations of sample location, type, quantity, and frequency will optimize identification of target microorganism(s). Regularly modified environmental sampling plans that can identify and predict the presence of *Listeria* and *L. monocytogenes* are an important way to prevent foodborne listeriosis.

P1-51 *Salmonella* spp. and *Listeria monocytogenes* in Minimally Processed Vegetables in São Paulo, Brazil: Incidence and Counts Data for Risk Assessment

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Introduction: Salmonella spp. and *L. monocytogenes* contamination in minimally processed vegetables (MPV) is of concern because of their association with outbreaks or ability to grow under refrigeration temperatures. Intervention strategies have been proposed to control or reduce their incidence; however, data on their counts in MPV are scarce, even being critical for risk assessment studies.

Purpose: This study aimed at quantifying and determining prevalence of *Salmonella* spp. and *L. monocytogenes* in MPV collected from retail establishments in the city of São Paulo, Brazil.

Methods: A total of 512 packages of MPV containing one or more types of vegetables were collected from supermarkets between April and August 2008. ISO methods for *Salmonella* spp. (ISO 6579) and *L. monocytogenes* (ISO 11290-1 e 11290-2) were used in the analysis. *Salmonella* spp. enumeration was done through an adaptation of ISO 6579 method.

Results: L. monocytogenes and *Salmonella* spp. were detected in 8 (1.6%) and 9 (1.8%) of the samples, respectively. Using ISO method 11290-2 it was possible to enumerate *L. monocytogenes* in 3 (0.6%) of the samples, and counts ranged between 1.0×10^1 and 1.7×10^2 $CFUg^{-1}$. The counts of *Salmonella* spp. were $\leq 10^1$ $CFUg^{-1}$ in all samples analyzed. Data indicated that seven (78%) of the nine positive samples for *Salmonella* spp. were composed of only one type of vegetable. On the other hand, five (62.5%) of the eight positive samples for *L. monocytogenes* were composed of more than two different types of vegetables. Samples in which enumeration of *L. monocytogenes* was possible were composed of more than four different types of vegetables.

Significance: Despite the low counts of *Salmonella* spp. and *L. monocytogenes*, data obtained are relevant for estimating the risks of salmonellosis and listeriosis associated with MPV consumption in storage conditions simulating both retail and consumer levels. This could lead to the establishment of science-based intervention strategies aimed at reducing the risks of these diseases.

P1-52 Alternative Sigma Factor σL Regulation is Important for Diverse Environmental Stress Responses in *Listeria monocytogenes*

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Introduction: Listeria monocytogenes is a foodborne pathogen of considerable public heath and food safety importance. This organism overcomes diverse stress conditions to grow on preserved food products and during host infection. Alternative sigma factor σL proteins are global transcription regulators implicated in control of various cellular processes in bacteria. Although *L. monocytogenes* σL functions are not yet fully understood, this protein presumably also contributes to the remarkable stress resistance of phenotypes observed in this bacterium.

Purpose: The present study was conducted to assess the contribution of σL in acclimation of *L. monocytogenes* to a wide range of environmental stress conditions.

Methods: Quantitative realtime qRT-PCR assays were first applied in order to investigate the influence of stress exposure on sigL (rpoN) gene expression. Logarithmic phase *L. monocytogenes* EGDe cultures adapted to growth under optimal (BHI 37°C) and stress conditions (BHI 10°C, BHI-2% NaCl and BHI-lactic acid pH 6.0) were analyzed. Secondly, we constructed an in-frame ΔsigL mutant in the EGDe strain genetic background. The growth and survival kinetics of this deletion mutant under different stress conditions were compared to those of the parental wild-type strain. Growth stress conditions included (1) cold temperatures (4 and 10°C), (2) elevated salt (DM-2% NaCl) concentration, (3) lactic acid (BHI-lactic acid pH 6.0) and iv) exposure to an oxidizing agent (BHI-6.5 mM cumene hydroperoxide). Survival was assessed under lethal lactic acid (pH 3.5) and oxidative (13 mM CHP) stress conditions.

Results: Significant stress-dependent induction of sigL gene expression was observed in wild-type *L. monocytogenes* EGDe cells exposed to cold (22-fold), NaCl salt (8-fold) and lactic acid (17-fold) stresses. Characterization of the ΔsigL deletion mutant revealed that although σL is not an essential gene, its functions are required for the efficient adaptation of *L. monocytogenes* to growth at low temperatures, and in the presence of elevated NaCl concetration and lactic acid stress environments. Interestingly, loss of σL was also associated with gain of function changes leading to increased growth and survival of *L. monocytogenes* cells under oxidative stress conditions.

Significance: This study has provided gene expression and genetic evidence in support of σL involvement in regulation of cold, osmotic, lactic acid and oxidative stress tolerance mechanisms of the foodborne pathogen *L. monocytogenes*.

P1-53 Effect of Growth and Recovery Temperatures on Pressure Resistance of *Listeria monocytogenes*

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Introduction: Experimental conditions can affect the outcomes of bacterial stress-tolerance studies. Conditions that optimize microbial recovery should be established to determine adequate treatment conditions for food safety.

Purpose: The objectives of this study were to determine the effects of growth and recovery temperatures on apparent pressure-resistance of *L. monocytogenes* in milk.

Methods: (1) *L. monocytogenes* was grown at various temperatures (10, 15, 20, 25, 30, 35, 40, 43°C) to early stationary phase, suspended in UHT-processed whole milk, pressure treated (400 MPa, 2 min, 21°C) and recovered on TSAYE at 35°C. (2) *L. monocytogenes* was grown at two temperatures (35 and 43°C), pressure treated in milk (400 and 500 MPa, respectively), and recovered on TSAYE at various temperatures (4, 10, 15, 20, 25, 30, 35, and 40°C). (3) *L. monocytogenes* was grown at 35°C, pressure treated in milk (400 and 450 MPa, 2 min, 21°C), and recovered on TSAYE at 10°C for various intervals (1, 2, 3, 6, 9, and 12 days) then at 35°C for 5 days. All studies were conducted in triplicate.

Results: (1) There was no significant difference ($P > 0.05$) in pressure resistance of *L. monocytogenes* grown at 10 to 25°C with approximately 6.5 log CFU/ml population reductions. Pressure resistance increased directly with higher growth temperatures; less than 1 log CFU/ml reduction was observed at 43°C. (2) Regardless of growth temperature and pressure treatment, the greatest recovery of *L. monocytogenes* was within 4 to 20°C by as much as 2 log CFU/ml greater than at other recovery temperatures; recovery at 10°C required 24 days. (3) The time for full recovery could be reduced by incubating at 10°C for 2 days then 35°C for 5 days.

Significance: Growth and recovery temperatures affect the apparent pressure resistance of *L. monocytogenes* and should be factored into the determination of adequate inactivation treatments.

P1-54 Impact of Affinity Purification on the Performance of Antibodies Specific for *Listeria* spp. and Their Use in a Multiplex Luminex Bead Array for Food Testing

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Introduction: Listeria monocytogenes is a major foodborne pathogen that causes the human disease listeriosis, which is associated with consumption of contaminated food products. Listeriosis results in a high mortality rate among immunosuppressed populations and a high rate of miscarriage in pregnant women. Immunoassay systems are efficient methods for early detection of *Listeria* spp. that fulfill most regulatory needs. However, rapid detection of *Listeria* using immunoassays is highly dependent on antibodies that have a high degree of sensitivity and specificity for the organism.

Purpose: The goal of this work was to investigate the importance of affinity purification in the sensitivity and specificity of anti-*Listeria* antibodies, using multiple immunoassay detection systems, particularly in a multiplexed assay system such as Luminex.

Methods: We purified goat anti-*Listeria* antisera both by standard Protein G chromatography and by affinity chromatography. The purified antibodies were tested in both ELISA and Western blot. Protein G-purified and affinity-purified antibodies were also both tested in a Luminex bead-array that was developed for the detection of five target organisms simultaneously.

Results: The affinity-purified antibody had a higher level of sensitivity and specificity than the Protein G purified antibody in both ELISA and Western blot. The two antibodies had similar performance in avidity assays. In the Luminex bead-array assay, both antibodies effectively detect *Listeria* among other organisms, with good specificity in a variety of food samples. Under these assay conditions, the affinity-purified anti-*Listeria* antibody outperformed the Protein G-purified antibody, but only when used in both capture and detection.

Significance: The results demonstrate the importance of using an affinity-purified antibody for *Listeria* detection in immunological tests. Furthermore, we have demonstrated the application of using this antibody in a multiplexed Luminex bead-array assay for the simultaneous detection of multiple food pathogens.

P1-55 Virulence for Mice, Resistance to Synthetic Gastric Fluid and Biofilm Formation of a Strain of *Listeria monocytogenes* Serotype 4b Isolated from a Listeriosis Outbreak Associated with Hot Dogs

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Introduction: One of the most severe listeriosis outbreaks occurred in 1998 to 1999 as a result of contamination of hot dogs with serotype 4b *Listeria monocytogenes*. There has been little characterization of virulence attributes of strains isolated from this outbreak.

Purpose: The purpose of this study was to compare several characteristics of strain H7550 isolated from vacuum-packaged frankfurters in the 1998 to 1999 outbreak of listeriosis, a plasmid-free derivative (H7550cds) of that strain lacking the cadmium resistance plasmid pLM80, and selected transposon mutant derivatives.

Methods: Virulence was assessed following intragastric (i.g.) inoculation of anesthetized A/J mice with approximately 10^6 CFU of the individual strains. We also compared the strains' resistance to inactivation in synthetic gastric fluid (pH 4.5), and ability to form biofilms on a plastic surface in vitro. A transposon mutant of strain H7550 that lacks adenylosuccinate lyase activity (strain J22F), and a non-hemolytic (LLO-) transposon mutant of strain H7550cds (J29H), were also included in our experiments.

Results: Comparable numbers of CFU were recovered from the spleen, liver, blood gallbladder, and ceca of mice inoculated with either strain H7550 or H7550cds. Mutants J22H and J29H were avirulent in our mouse model; we did not recover viable cells from any of the internal organs. We observed no significant difference in the resistance of stationary phase cells of the plasmid-harboring versus plasmid-free strains to synthetic gastric fluid at pH 4.5. Mid log phase cells of mutant strain J22H appeared to be more resistant to inactivation at pH 4.5 than mid log phase cells of the other strains examined. Strains H7550 and H7550cds both formed biofilms on plastic surfaces within 24 h in vitro. Strain J22F was better able to form biofilms in vitro than its parent strain H7550, whereas the LLO-negative mutant strain J29H was not significantly different from its parent strain H7550cds in biofilm formation.

Significance: Strain H7550 and its cadmium sensitive derivative H7550cds are equally virulent for mice, and are both resistant to synthetic gastric fluid, but they may exhibit minor differences in the ability to form biofilms in vitro. LLO is required for virulence of this strain, as for other *L. monocytogenes*, but was not required for biofilm formation. Transposon mutant strain J22F was also avirulent for mice, providing the first evidence that adenylosuccinate lyase activity is required for virulence in mice. Further studies are needed to assess the possible role of pLM80 in fitness of the bacteria associated with the 1998 to 1999 outbreak.

P1-56 Genotyping of *Listeria monocytogenes* Isolated from the Environment and Food Products in a Convenience Food Processing Plant

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Introduction: L. monocytogenes as a foodborne pathogen has significant public health and economic impacts. The infection of humans may result in severe clinical disease as well as high mortality. Furthermore, *L. monocytogenes* contamination is one of the leading causes of recalls in processed foods. Among other foods, Ready-to-Eat products have been implicated as routes for human infection.

Purpose: The aim was to investigate the occurrence and genetic diversity of *L. monocytogenes* strains from a convenience food-processing plant.

Methods: Samples were obtained twice a week from the processing room, from machines and equipment of five sandwich production lines, and from products. Isolation of *Listeria* was accomplished by culture after enrichment (Half-Fraser/Fraser broth and Palcam agar). For identification of *L. monocytogenes*, suspicious colonies were streaked onto Ottaviani Agosti agar. Genetic lineages were determined using the ASO-PCR multiplex system targeting the *prfA* virulence gene cluster. Moreover, strains were genotyped by REP-PCR to establish genetic relationships.

Results: Of the 500 samples, 6.0% were positive for *L. monocytogenes*. Nearly half the positive samples (46.7%) originated from three slicers. Besides, five sandwiches (twice tuna; once each salmon, egg, and ham) tested positive, and *L. monocytogenes* were also isolated from tables, gloves, the floor, a conveyor belt, a spattle, a squeegee, and a water hose. Of the 30 isolated strains, 28 (93.3%) grouped into genetic lineage II, whereas two strains from the water hose belonged to lineage I. REP-PCR grouped the 30 strains into three different genotypes. Twenty-five (83.3%) strains belonged to only one genotype. On two slicers and a table, *L. monocytogenes* belonging to the dominating genotype were isolated in repeated samplings.

Significance: Genotyping results indicate the predominance and persistence of a certain *L. monocytogenes* strain in the processing area of this convenience food-processing plant, even after cleaning and disinfecting procedures. Thus the slicing equipment, which is used for different products and also at different production lines, presents a possible source and risk factor for product contamination with *L. monocytogenes*.

P1-57 Survival of Desiccated *Listeria monocytogenes* on Stainless Steel and Transfer to Salmon Products

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Introduction: Listeria monocytogenes is a Gram-positive foodborne bacterial pathogen capable of colonizing food processing equipment and cross-contaminating processed foods, potentially causing foodborne listeriosis. A recent example is the 2008 Canadian outbreak from cross-contaminated meat products. The persisting capacity of *L. monocytogenes* is not fully explained. Food processing environments experience constant changes in relative humidity (RH), and contaminating microorganisms may be subjected to low RH conditions for extended periods of time.

Purpose: The objective of this study was to examine the survival on stainless steel and subsequent transfer to salmon products of desiccated *L. monocytogenes* N53-1, a persistent food strain.

Methods: During desiccation on stainless steel coupons at 43% RH and 15°C, survivors were enumerated by spread plating on BHI and Oxford agar. The physiological state of desiccated cells was observed by use of fluorescence microscopy with the LIVE/DEAD stain.

Results: The survival of cells (initial count 7.7 log CFU/cm^2) desiccated for 23 days in presence of 5% (w/v) NaCl was significantly ($P < 0.05$) higher (6.8 log CFU/cm^2) than the survival of cells desiccated with 0.5% (w/v) NaCl (5.4 log CFU/cm^2). LIVE/DEAD fluorescence staining showed dominance of healthy (green) cells in 5% NaCl samples while damaged/dead (red) cells dominated 0.5% NaCl samples. Allowing *Listeria* to form biofilm (2 d at 100% RH and 15°C) prior to desiccation improved the survival of 0.5% NaCl samples significantly, indicating the protective effect of the biofilm matrix. Also, cells pre-cultured in 5% NaCl before the biofilm/desiccation treatment survived significantly ($P < 0.05$) better than cells pre-cultured in 0.5% NaCl. The desiccated bacteria transferred easily from steel to smoked and fresh salmon, and transfer was proportional to the numbers of live cells on the steel coupons.

Significance: In conclusion, we have shown that *L. monocytogenes* survived desiccation well, that the presence of salt during preculture and desiccation increased survival and that this in turn enhanced the transfer of bacteria to foods.

P1-58 Growth of *Listeria monocytogenes* in Thawed Frozen Foods

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Introduction: FDA released a draft Compliance Policy Guide (CPG) on *Listeria monocytogenes* and draft guidance to industry in February 2008. FDA has proposed that Ready-to-Eat foods that do not support the growth of *L. monocytogenes* may contain up to 100 CFU/g. Frozen foods fall in that category, but once they are thawed, held refrigerated and consumed without further cooking, growth of *Listeria* may present a health hazard.

Purpose: The purpose of this study was to develop data that demonstrates how *L. monocytogenes* grows on foods that were previously frozen and then thawed and held at refrigeration temperatures, such as on a salad bar.

Methods: Twelve strains of refrigeration temperature-adapted *L. monocytogenes* were used to inoculate four frozen food samples: corn, green peas, cooked crab meat, and cooked shrimp. Growth of *L. monocytogenes* was monitored at four different temperatures (4, 8, 12 and 20°C) using the plate count method with PALCAM agar. In addition, aerobic plate counts on non-inoculated samples and informal sensory analysis on inoculated samples and non-inoculated samples were conducted at the same time at all temperatures, to observe the relationship between product spoilage and growth of *L. monocytogenes*.

Results: Lag phase and exponential growth rates were calculated for all conditions using the Gompertz model. *L. monocytogenes* tended to grow faster on corn and green peas than on crab meat and shrimp at 4, 8 and 12 °C during storage. All samples showed low initial aerobic plate counts, but counts on corn, green peas, and shrimp increased rapidly at all temperatures.

Significance: The data developed can possibly be used by FDA to develop guidance on enforcement criteria and possibly in the Food Code on how to handle these types of foods.

P1-59 Adaptation of the Lateral Flow Immunochromatographic Hand-held System for the Detection of *Staphylococcal enterotoxin* B (SEB) or *Staphylococcal enterotoxin* C (SEC) in Commercial Infant Formulas, Baby Foods, Milk and Milk Products

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Introduction: Rapid, sensitive, and specific methods have been developed to detect either SEB or SEC contamination in a variety of dairy products. Further, methods were developed to extract and detect SEB in baby foods, which were texturally complex and varied with respect to ingredients and colors.

Purpose: The purpose of this study was to provide adaptive techniques that are fast and easy to use for the qualitative detection of toxin in wide variety of foods used by a large and sensitive group, such as infants and very young children.

Methods: Two lateral flow immunochromatographic assay devices (LFDs), developed by the Department of Defense for the detection of environmental contamination with SEB or SEC, were adapted for use in a variety of infant formulas and foods, milk and popular commercial dairy products. Three concentrations of SEB or SEC (5 µg/ml, 500 ng/ml, and 5 ng/ml) were used. 1 ml volume of toxin or Phosphate buffered saline (PBS) was added to the food matrices. The semi-solid food matrices were mixed thoroughly following the addition of 19 mls PBS. All samples were incubated for 2 h at 4°C, then centrifuged (1500 × g 20 min). One-hundred fifty µl of the supernatant was applied to the lateral flow devices.

Results: Once the product wicked onto the strip, either toxin was easily detectable as far as 5 ng/ml. 2% milk and milk-based infant formula, wicked easily into the lateral flow devices strips. Soy-based formulas, chocolate milk, and ice creams required dilution to wick into the LFDs. There were no discernable differences in the ability to detect the three concentrations of either toxin regardless of color, texture or the type of ingredient used to make the ice-cream or the baby foods. The inability to detect the last dose in baby foods may have been a function of the degree to which the sample was diluted in order to induce sample flow onto the strip. SEC was not tested with baby food items.

Significance: The lateral flow immunochromatographic device is fast, accurate, and adaptable to a wide variety of food matrices. The ease of use and assay, coupled with simpler extraction strategies, would permit rapid and reliable detection of SEC or SEB in these foods.

P1-60 DSC Inactivation of *Escherichia coli* O157:H7, *L. monocytogenes*, *Salmonella enterica* and *Shigella flexneri* on Iceberg Lettuce (*Lactuca sativa*) by X-ray

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Introduction: Vegetables are an important part of healthy eating. However, vegetables, including iceberg lettuce, have been associated with many foodborne outbreaks. X-ray technology is a promising non-thermal method of reducing pathogenic and spoilage bacteria on food.

Purpose: The purpose of this investigation was to study: (a) efficacy of X-ray doses (0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 1.5 and 2.0 kGy) against inoculated *E. coli* O157:H7, *L. monocytogenes*, *Salmonella enterica* and *Shigella flexneri* on iceberg lettuce, and, (b) the effect of X-ray treatment on the quality and shelf life of iceberg lettuce during storage at 4°C.

Methods: 100 µl of each targeted organism was spotted onto the surface (10 cm^2) of lettuce leaves (approximately 9 log ml-1) separately, after which the leaves were air dried and treated with X-ray at 22°C and 55% relative humidity. Surviving bacterial populations were evaluated using a non-selective medium (Tryptic Soy Agar) with the appropriate selective medium overlay for each organism; for *E. coli* O157:H7 (CT-SMAC agar), for *L. monocytogenes* (MOA), and for *Salmonella enterica* and *Shigella flexneri* (XLD).

Results: More than 5 log CFU reduction (recommended by FDA) of E. coli O157:H7, *L. monocytogenes*, *Salmonella enterica* and *Shigella flexneri* was achieved with 2.0 kGy X-ray. Treatment with X-ray significantly reduced the initial microflora on iceberg lettuce and kept them significantly ($P < 0.05$) lower than the control during storage at 4°C. Furthermore, treatment with X-ray did not significantly ($P > 0.05$) change the green color of iceberg lettuce.

Significance: These results showed that X-ray treatment significantly reduced selected pathogens and inherent microorganisms on iceberg lettuce, which suggests a good alternative to other technologies for the fresh produce industry.

Poster

P1-61 Assessing the Use of Specific Cooling Practices to Prevent *Clostridium perfringens* Growth in Refried Beans

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Introduction: Since 1990, *Clostridium perfringens* has been reported as the causative agent in fifty-seven foodborne disease outbreaks (3,636 cases), with 16 of these outbreaks (1,654 cases) related to refried beans. This high incidence is due to survival of the bacterium spores during cooking and subsequent germination and multiplication due to improper cooling, heating, or storage.

Purpose: We conducted cooling experiments on refried beans. We predicted growth of *C. perfringens* in refried beans cooled using actual practices followed in some Mexican restaurants and compared it to the growth of *C. perfringens* in refried beans cooled based on FDA Food Code practices.

Methods: In three replications, refried beans were prepared according to a standard preparation process using pinto beans (453 g beans per 1L water) with and without salt (2.5%). The beans (3.63 kg) were cooled in either a 22 qt stock pot or a shallow pan (24 × 16 × 1 in.) at refrigeration temperatures (5°C). Refried bean temperatures were recorded until the temperature reached 5°C. Perfringens Predictor was utilized to determine the growth of *C. perfringens* during the cooling of the refried beans.

Results: Refried beans cooled in the 22 qt stock pot reached the desired temperature of 5°C in 6 h to 12 h longer than the FDA recommendations. Growth of 1.23 log CFU/g and 0.91 log CFU/g of *C. perfringens* was predicted in beans without and with 2.5% NaCl, respectively. The beans cooled in a shallow pan reached 5°C within 3 h. Salt (2.5%) did not affect the growth of *C. perfringens* because of proper prevention of the growth of *C. perfringens* by proper cooling.

Significance: Improper cooling practices for refried beans create conditions that may result in the growth of *C. perfringens*; proper cooling protocols will prevent the growth of *C. perfringens* and subsequent foodborne disease outbreaks.

P1-62 First Report on *cpe*-positive Type A *Clostridium perfringens* from Food Samples in the State of São Paulo, Brazil

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Introduction: Clostridium perfringens is an important foodborne disease agent. However, the detection of this species in foods, even in those suspected to have caused food poisoning, is not sufficient to consider the isolates a risk to the health of the consumers. Among the five types of *C. perfringens* recognized (A to E), type A is responsible for the majority of human illnesses. Around 1 to 5% of *C. perfringens* type A isolates produce *C. perfringens* enterotoxin (CPE), a clinically important toxin.

Purpose: Identify the frequency of *C. perfringens* among sulfite-reducing *Clostridia* isolates, characterize the toxin type of *C. perfringens* and determine the presence of the *cpe* gene.

Methods: 200 sulfite-reducing *Clostridia* isolates (116 from food; 84 from soil), from samples collected in the State of São Paulo, Brazil, were analyzed. The isolates were submitted to biochemical tests for *C. perfringens*, identification, and to multiplex PCR for toxin genotyping. *cpe* evaluation was conducted by simplex PCR.

Results: 66 (56.9%) of the food and 43 (51.2%) of the soil isolates were identified as *C. perfringens*, all being typed as *C. perfringens* type A. Twenty *C. perfringens* isolates were *cpe*-positive: 19 were from foods suspected to be linked to foodborne outbreaks, and one from other food origin. None of the soil isolates harbored *cpe*.

Significance: The use of sulfite-reducing *Clostridia* as an indicator of *C. perfringens* is recommended by Brazilian regulations. Our results show a low correlation between them. Although the detection of *cpe* was almost exclusive to outbreak-related food, this is the first report in the country of food origin *C. perfringens* harboring this gene. The location of the *cpe* gene will be evaluated. Soil might not be the major reservoir of *C. perfringens* that is able to cause food poisoning.

P1-63 Extracellular Protectants Produced by *Clostridium perfringens* Cells at Elevated Temperatures

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Introduction: The mechanisms of adaptation of *Clostridium perfringens* to high temperatures are not well understood.

Purpose: In this work, the involvement of extracellular compounds in protection to heat was determined.

Methods: Cells were grown in fluid thioglycollate medium or chicken broth. When mid log phase was reached, they were heat shocked at 50°C for 30 min. Then cultures were centrifuged and supernatants were transferred to non-shocked cells. Heat tolerance of these cells was performed at 55°C. Viable cells were determined. In some cases, supernatants were heated at 65 or 100°C or treated with trypsin. Supernatants were fractionated and PAGE was made of fractions showing heat-protective activity.

Results: When *C. perfringens* was exposed to a heat shock at 50°C, extracellular factors were found in the culture supernatant that provided protection to cells not exposed to a heat shock. The extracellular factors were sensitive to heat and trypsin treatment, suggesting a protein component. SDS-PAGE analysis of supernatant fractions from heat-treated cells revealed two induced proteins (56 and 125 kDa) that could be involved in heat tolerance.

Significance: In this work, the presence and thermoprotective activity of extracellular factors produced by *C. perfringens* under a heat shock was demonstrated. The detection of thermoprotective extracellular factors of *C. perfringens* will aid in our understanding of the physiology of survival of *C. perfringens* in foods.

P1-64 Molecular Characterization of Multidrug-resistant *Clostridium difficile* Isolated from Wild Pigs, Production Pigs and Humans

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Introduction: Clostridium difficile-associated disease (CDAD) is the most common cause of nosocomial diarrhea. The recent detection of *C. difficile* Toxinotype V in retail meat products, including pork, has pointed out the importance of determining the environmental reservoirs which may act as sources of transmission to food animals and humans.

Purpose: The objective of this study was to characterize and compare the toxinotypes and antibiotic resistance profiles of *C. difficile* isolated from feral swine, production swine, and humans in North Carolina.

Methods: A total of 83 isolates comprised of 21 from feral swine, 29 from production swine and 33 from humans were characterized. All the isolates were characterized for their toxinotype profile. We determined their antimicrobial resistance profile to a panel of eight antimicrobials using the Epsilometric test. Genes associated with resistance to tetracycline (tetM and tetW) and erythromycin (ermB, ermF, and ermQ) were amplified. The gyrA gene, associated with floroquinolone resistance, was sequenced to determine presence of mutations.

Results: Toxinotype V was the predominant pattern identified in both the feral (90%) and production pigs (93%). The human isolates represented a wide variety of toxinotypes, with toxinotype 0 (45%), III (12%) and XII (18%) being predominant. Tetracycline resistance was found only in production and feral pigs with the tetM and tetW alleles coding for the above phenotype. Macrolide resistance was observed predominantly in the human isolates (42%), with only one feral isolate exhibiting resistance. Sixty percent of the isolates carried the ermB gene. All isolates were resistant to ciprofloxacin, while only the human *C. difficile* isolates exhibited resistance to gatifloxacin (21%). Point mutations in gyrA gene indicated non-synonomous amino acid changes in the human isolates.

Significance: Identical toxinotype V and resistance profile shared between the feral and production swine indicates the presence of environmental reservoirs of *C. difficile*. Recent reports indicate that prevalence of toxinotype V is increasing in humans. It is, therefore, important to monitor the transmission potential of *C. difficile* through environmental reservoirs to food animals and retail meats, to safeguard public health.

P1-65 Thermal Resistance of *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Yersinia pestis* Grown at Two Different Temperatures

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Introduction: Yersinia enterocolitica and *Yersinia pseudotuberculosis* are foodborne pathogens. Although *Yersinia pestis* is not considered a foodborne pathogen, it has caused illness through food consumption and could be a target of intentional food contamination. Although the thermal resistance of *Y. enterocolitica* has been studied, limited information is available on the thermal resistance of *Y. pseudotuberculosis* and *Y. pestis*.

Purpose: To determine the thermal resistance of different strains of *Y. pseudotuberculosis* and *Y. pestis* and compare their resistance to the better known *Y. enterocolitica* under two growth conditions which allow the expression of different virulence states.

Methods: One strain of *Y. enterocolitica*, three strains of *Y. pseudotuberculosis*, and two strains of *Y. pestis* were grown individually at 25 and 37°C, harvested, and resuspended in pH 7 buffer. Samples were loaded into capillary tubes, sealed, and treated at different temperatures for various time intervals. Survivors were enumerated.

Results: Y. enterocolitica exhibited 4-fold greater heat resistance than all strains of *Y. pseudotuberculosis* and *Y. pestis* when grown at 37°C and 2-fold greater thermal resistance when grown at 25°C. As opposed to *Y. enterocolitica, Y. pseudotuberculosis* did not display greater thermal resistance when cultivated at 37 rather than 25°C. The most thermal resistant strain of *Y. pseudotuberculosis* (Y197), had a calculated D_{54}= 1.32 ± 0.11 min, with a z-value of 3.97 when grown at 25°C and a D_{54}= 1.1 ± 0.03 min, z = 3.39 when grown at 37°C. The thermal resistance of *Y. pestis* was similar to that calculated for *Y. pseudotuberculosis* (D_{54}= 1.07 ± 0.35 min, z = 3.92 when grown at 25°C).

Significance: Y. enterocolitica was determined to be more heat resistant than either *Y. pseudotuberculosis* or *Y. pestis*. All strains displayed less thermal resistance than other typical foodborne pathogens such as *Escherichia coli* or *Salmonella*.

P1-66 Phenotypic, Genotypic and Serotypic Assessment of Virulence Traits and Antibiotic Susceptibility of *Yersinia enterocolitica* Isolated from US Market Weight Hogs

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Introduction: Yersinia enterocolitica (YE) causes an estimated 96,000 cases of human gastrointestinal disease annually in the United States. Swine are the only known animal reservoir of YE pathogenic to humans.

Purpose: Since YE is an enteric commensal of pigs, the primary goal of this study was to characterize YE swine fecal isolates by phenotype, including serotype and antibiotic susceptibility, and genotype by PFGE analysis.

Methods: Swine fecal samples (n = 2,793) collected from 78 production sites in 15 major pork-producing states across the United States were tested for the presence of YE in pigs. YE were isolated using a combination of ITC medium for enrichment and CIN agar and were identified by fluorogenic 5' nuclease PCR targeting the chromosomal attachment-invasion ail locus. Isolates were initially tested for markers of virulence including carriage of a 70-kbp plasmid, colony morphology, low calcium response, Congo red uptake, crystal violet binding, autoagglutination, hydrophobicity, and presence of a cytotoxicity factor, YopE. For phenotype characterization, serotyping and antimicrobial susceptibility testing were performed. Genomic analysis of the isolates was determined by PFGE.

Results: In this study, 106 ail-positive YE were isolated from 2,793 fecal samples. One isolate from each positive sample was characterized. The presence of virulence plasmid, expression of plasmid-associated virulence determinants, and serotype were correlated with genotype, expression of YopE protein and antibiotic susceptibility. All isolates contained the virulence plasmid and expressed virulence-associated phenotypic characteristics. The 25kD YopE protein was present in 104 of 106 (98%) of isolates. The 106 ail-positive isolates were identified as either serogroup O:3 (74.5%) or O:5 (25.5%). PFGE showed that O:3 and O:5 isolates were highly clonal within a serotype regardless of geographic origin. Antimicrobial resistance profiles of 106 isolates of serotypes O:3 and O:5 showed that all isolates were susceptible to 13 of the 16 tested antimicrobials; resistance was noted to ampicillin, cephalothin, and tetracycline.

Significance: The presence of the ail gene, virulence plasmid, the expression of virulence determinants and serotypes indicate that these isolates are potentially capable of causing human foodborne illness. Additionally, the results confirmed that US swine are a reservoir for pathogenic YE.

P1-67 Effect of Cooling Rates on Survival and Growth of *Escherichia coli* O157:H7 in Creamed Cottage Cheese

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Introduction: Escherichia coli O157:H7 has been demonstrated to have tolerance to lower pH values, such as in cheese curds, and can grow at temperatures from 8 to 45°C. The US Pasteurized Milk Ordinance (PMO) utilizes a dairy product fill and storage requirement of 7.2°C; however, the dairy industry has proposed a modification to 12.8°C, cooling to 7.2°C within 72 h.

Purpose: This study was to compare the survival and growth of acid-adapted *E. coli* O157:H7 in full-fat (4%) and reduced-fat (1%) creamed cottage cheese at the PMO-required temperature of 7.2°C versus the same product utilizing natural microbial inhibitors, inoculated at 12.8°C and cooled to 7.2°C within 72 h.

Methods: Cottage cheese was formulated with 55% dry curd and 45% cream dressing supplemented with microbial inhibitors derived from natural sources. The pH of the product was adjusted to target pH 5.2. Treatments included control (no antimicrobials), 0.03% and 0.04% Bioactive Protein I, 0.15% Fermentate D, 0.1% Fermentate E with and without live culture, and 0.3% and 0.5% Fermentate A. The inhibitors could be characterized as cultured or fermented milk solids, calcium lactate or naturally derived from milk. Inoculated products were cooled from 12.8°C to 7.2°C either immediately (PMO cooling requirement) or within 72 h(according to industry practices).

Results: Cottage cheese with Inhibitors A, D and E at all concentrations performed as well as or slightly better than the control at day 14, while Inhibitor I caused a 3 log reduction in *E. coli* O157:H7 at day 14.

Significance: This study demonstrated that at all fat levels of cottage cheese (initial pH 5.2), the addition of natural microbial inhibitors, either before or after pasteurization, will reduce the growth of *E. coli* O157:H7 for up to 14 days during the industry-proposed "slow" cooling at an equal or greater rate compared to the current regulatory requirement of 7.2°C.

P1-68 Pathogen Presence and Levels of Generic *Escherichia coli* during Turned Pile Composting of Broiler Litter

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Introduction: Recent produce outbreaks highlighted the importance of expanding knowledge regarding pathogen presence in agricultural environments. Composted manure represents an important nutrient source for organic production.

Purpose: Indicator organism levels and pathogen presence during turned pile (TP) composting of broiler litter was examined.

Methods: To meet requirements for the process to further reduce pathogens (PFRP), the TP must reach 55°C, and that temperature must be maintained for at least 15 days, during which time the TP must be turned 5 times. Prior to composting and at each turning, the TP was sampled in 5 locations at three depths (surface, 30 cm, 60 cm) and 3 samples were collected immediately after turning. Samples were quantified for generic *E. coli* and for the presence of *E. coli* O157:H7 and *Salmonella*.

Results: Prior to composting, both *Salmonella* and *E. coli* O157:H7 were detected at the surface and at 30 cm, respectively; *E. coli* levels were consistent (0.30 log CFU/ml) at all 3 depths at all 5 sampling locations. *E. coli* O157:H7 was detected after the first turn and prior to the second turn (30 cm and 60 cm). No pathogens were detected prior to the third turn. *Salmonella* was detected at 30 cm prior to the fourth turn, and *E. coli* O157:H7 was detected immediately after the fourth turn. No pathogens were detected after the fifth turn, and the TP met PFRP time and temperature requirements. Generic *E. coli* levels at the surface and within the pile remained stable at 0.30 log CFU/ml throughout the sampling periods; increases were observed immediately after the first two turns (0.82 and 2.57 log CFU/ml) but remained stable after the remaining turns (0.30 log CFU/ml).

Significance: Pathogens were detected during the composting process in samples that had corresponding low levels of generic *E. coli*. The TP compost met PFRP requirements, and pathogens were not detected in the finished compost.

P1-69 Influence of Modified Atmosphere Packaging (MAP) on *Escherichia coli* O157:H7 Growth, Survival, Shiga-toxin Production and Biofilm Production

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Introduction: MAP technologies in the food industry improve shelf life and sensory characteristics while providing some microbial control. Studies indicate that pathogens may be inhibited under certain MAP conditions thus providing a measure of food safety.

Purpose: This study was conducted to observe cell growth and survival, production of shiga toxins, and biofilm formation by *Escherichia coli* O157:H7 under various MAP conditions.

Methods: 25 ml aliquots of a 5 log CFU/ml and 1.5 log CFU/ml culture of *E. coli* O157:H7 ATCC 43895 prepared in Tryptic Soy Broth (TSB) were transferred, aseptically, into culture flasks for incubation at 4°C and 25°C, respectively. Flasks were packaged under four conditions; high oxygen (HIOX; 80% O_2/ 20%CO_2), with carbon monoxide (CO; 0.4% CO/ 35% CO_2/64.6% N_2), without carbon monoxide (without CO; 70% N_2/30% CO_2) and overwrap (OW). Flasks were sampled on days 0, 5, 10, 15 and 20 for viable cell counts, production of shiga toxins, as well as biofilm formation on day 20 only. Additionally, the same design was used to observe growth curve data and pH, for the 1.5 log CFU/ml inoculated 25°C samples at 0, 4, 8, 12, 16, 20, 24, 36, 48 and 72 h.

Results: CO and Without CO 4°C samples decreased to 3.28 and 3.65 log CFU/ml, by Day 20. HIOX and OW 4°C samples were not detectable by Day 20, indicating a 5 log decrease. Toxin production was negative for all 4°C samples. HIOX, CO, Without CO, and OW samples at 25°C increased to 9.53, 9.08, 9.22 and 9.44 log CFU/ml by Day 5 and to 9.41, 9.06, 9.06 and 8.90 log CFU/ml by Day 20, indicating no significant inhibition among treatments. All 25°C samples were negative at Day 0 for toxins and positive for both toxins 1 and 2 at Days 10 and 20. Biofilm formation was observed by light microscopy and scanning electron microscopy (SEM). Images indicated multi-layer film formation for HIOX and OW and reduced formation for CO and Without CO samples at 25°C. Growth curve samples for 25°C indicated the greatest inhibition of O157 proliferation in the HIOX packages, followed by CO and Without CO treatments, and the least amount of inhibition in the OW packages. By hour 8, an average .93 log CFU/ml difference was observed between OW and HIOX treatments which increased to approximately 1.57 log CFU/ml at hour 20 and approximately 1.85 log CFU/ml at hour 24. CO and Without CO treatments were largely indistinct at all time points. By hour 72, all treatments appeared to reach stationary phase at approximately ± 9.5 log CFU/ml. Measurements of pH for HIOX increased from approximately 6.8 at h 0 to 7.5, which remained constant through hour 24. HIOX, Without CO, and CO all remained at an approximate pH range of 6.0 to 6.5 through hours 24 and 36. All treatments exhibited a drop in pH between the hours of 24 and 36 which ranged from 1 pH unit to approximately 1.5 units, which would then return to previous measurements by hour 72. OW, CO, and Without CO treatments all tested positive for SLT-2 at hour 36, and all treatments were negative for SLT-1. At hour 48 all treatments tested positive for both SLT-1 and SLT-2.

Significance: These data suggest MAP packaging atmospheres may influence physiological characteristics of *Escherichia coli* O157:H7 at 4°C and 25°C in liquid media.

P1-70 Detection of Sorbitol Utilization, Virulence Genes and Intimin Types of Verotoxin-producing (VTEC) *Escherichia coli* Isolated from Food, and from Veterinary and Clinical Sources

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Introduction: Verotoxin-producing *Escherichia coli* (VTEC) have emerged as a leading cause of foodborne disease outbreaks in the United States. This group of bacteria poses a serious threat to public health as it is capable of causing increased morbidity and mortality.

Purpose: The objectives of the study were to detect sorbitol fermentation and the presence of the virulence genes and intimin types from food, veterinary and clinical sources.

Methods: Two hundred isolates were analyzed, using modified tryptic soy broth with novobiocin as pre-enrichment broth and cefixime-tellurite Sorbitol MacConkey Agar (CT-SMAC) as a selective media. The isolates were characterized for sorbitol fermentation, the presence of verotoxins (VT-1 and VT-2) and Intimin (*eae*-all); subtypes *eae*-α, *eae*-γ, *eae*-μ, *eae*-ε and *eae*-κ by multiplex PCR.

Results: Of the 200 isolates analyzed, one hundred fifteen were positive for *E. coli*. Fifty-eight strains were isolated from food samples, 27 from veterinary samples and 30 from clinical samples. Eighty of the 115 isolates (70.0%) were non-sorbitol fermenters. Using multiplex PCR, 83 (72%) were positive for the intimin (eae-all) gene; 63 (55%) were positive for eae-γ, no sample was positive for the other intimin subtypes. In addition, 102 (89%) of the isolates were VT 1 positive and 80 (70%) were VT 2 positive. Of the 58 strains isolated from food, 47 (81%) were positive for intimin (eae-all) 55 (94%) for VT 1 and 49 (84%) for VT 2 genes. The veterinary isolates were all negative for eae-γ while only 13 (48%) were positive for intimin. One-hundred percent of the veterinary isolates were positive for VT 1 and 13 (48%) were positive for VT 2 gene. Nineteen out of 30 clinical isolates (63%) were positive for all intimin subtypes genes and 20 (67%) and 18 (60%) were positive for VT-1 and VT-2 genes, respectively. In general, lowest percentages of genes and sorbitol fermenters were found in the veterinary source, while the highest percentages of genes and non-sorbitol fermenters were observed in the food samples.

Significance: These results indicate that there is widespread distribution of potentially virulent *E. coli* strains in food, veterinary and clinical sources that may be a cause of concern for human health.

P1-71 Comparison of Expression of *Escherichia coli* O157:H7 Virulence Factors in Ground Beef and on Fresh-cut Lettuce

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Introduction: Several *E. coli* O157:H7 outbreaks associated with leafy greens resulted in high rates of hospitalization and incidences of hemolytic uremic syndrome in patients, indicating that *E. coli* O157:H7 may be more virulent on leafy greens than in ground beef.

Purpose: The objective was to determine if the expression of *E. coli* O157:H7 virulence factors is different on cut lettuce than in ground beef by measuring gene expression on both commodities.

Methods: *E. coli* O157:H7 was inoculated onto lettuce, or ground beef, or into TSB and incubated at 37°C for 24 h. RNA from these cells was extracted. Reverse-transcriptase real-time PCR was used to evaluate expression of six virulence genes (*stx*II, eae, ehx, espA, ihaA, and rfbE) and one standard gene (gnd) in ground beef, on lettuce, or in TSB. Cycle threshold counts of each gene in TSB were used to construct standard curves, and expression of genes from cells grown on lettuce or ground beef were fitted to the curve. These values were measured against expression of gnd on lettuce or ground beef, and ratios were analyzed for significant differences ($P < 0.05$) in expression of genes based on commodity.

Results: The expression of virulence factors rfbE (O157-antigen) and ehx (hemolysin) were significantly higher when *E. coli* O157:H7 was grown in ground beef than on cut lettuce. No differences in expression of *stx*II (shiga toxin 2), eae (intimin), espA (type III secretion filament), or ihaA (adherence factor) were observed when *E. coli* O157:H7 cells were grown on lettuce or ground beef.

Significance: These data indicate that the growth environment provided by iceberg lettuce supported statistically similar levels of expression of four of six virulence factors of *E. coli* O157:H7 as ground beef, indicating that the growth environment may not be the sole basis for severe illness observed in previous outbreaks associated with consumption of leafy greens.

P1-72 Heat Resistance of Seven Pathogenic STEC Serotypes, Including O157:H7, in Single Strength Apple Juice

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Introduction: Without diminishing the importance of *Escherichia coli* O157:H7 as a major cause of disease worldwide, several researchers showed that non-O157 STEC serotypes make a significant contribution to the overall disease burden. The number of human illnesses due to non-O157 Shiga toxin-producing *E. coli* (STEC) infection has increased in the past decade worldwide. Pathogenic non-O157 STEC may pose a significant risk to public health. Current interventions for STEC in processed food are based on *E. coli* O157:H7. To date, little research has focused on the control of non-O157 STEC in foods.

Purpose: To determine the heat resistance of six non-O157 STEC serotypes relative to *E. coli* O157:H7, as a means of assuring adequate processing parameters for the new pathogen in single strength apple juice.

Methods: The heating data was obtained using an immersed coil apparatus, equilibrated at 56, 60, and 62C. Inoculum concentration in juice, prior to inactivation, was approximately 10^6 to 10^7 CFU/ml. Heat-treated samples (500 µl) were collected at timed intervals in glass vials, immediately cooled in an ice water bath, diluted using 0.1% peptone, and plated on TSA with a spiral plater. Inoculated plates were incubated for 48 h at 35°C. Colony counts were obtained using the Q count system.

Results: While serotype O26 presented the lowest D-values at all temperatures (D56C = 2.5 min; D60C = 0.52 min and D62C = 0.28 min), serotypes O145 and O45 revealed the highest D56C (4.7 min and 4.3 min, respectively) amongst the six non-O157 serotypes studied, although it was significantly lower ($P < 0.05$) than that of *E. coli* O157:H7 (D56C = 8.51 min) in the same conditions. At 60°C *E. coli* O157:H7 and O103 presented the highest D-values (0.99 and 0.94 min respectively), followed by O45 and O145. In general, *E. coli* O157:H7 revealed similar or higher D-values than the six non-O157 STEC serotypes studied in apple juice.

Significance: Present data show that the interventions that address *E. coli* O157:H7 in apple juice would be effective against non-O157 STEC as well.

P1-73 Translocation of *Escherichia coli* O157:H7 during Needle Injection for Moisture Enhancement of Meat DSC

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Introduction: Outbreaks linked to consumption of nonintact meat products contaminated with *Escherichia coli* O157:H7 indicate that processing may introduce pathogens into the interior of the meat. Such contamination may survive if the product is undercooked.

Purpose: This study evaluated quantitative transfer of *E. coli* O157:H7 during moisture enhancement of beef under two contamination scenarios.

Methods: In the first contamination scenario, beef knuckles (approximately 4 kg) were surface-inoculated (5 log CFU/g) with nonpathogenic rifampicin-resistant *E. coli* O157:H7 (5-strain mixture). The meat was then injected, with a hand operated single-needle brine injector, with either sterile distilled water (control) or a brine solution (NaCl [5.5%] and sodium tripolyphosphate [2.75%]) at seven locations per knuckle. In the second contamination scenario, the water and brine solution were inoculated (3 to 4 log CFU/ml) with *E. coli* O157:H7 and these were used for needle injection. Knuckles were weighed before and after injection and percent increase in weight was determined. One core (8 cm diameter) sample per knuckle was excised parallel to the direction of needle injection, using a coring device. Core samples were surface-decontaminated with hot water (80°C, 60 s), cooled (4°C, 15 min), and cut into six sections (1 through 6) of 1-cm (sections 1 through 3), 2-cm (sections 4 and 5), and 3- to 10-cm (section 6) thickness, while keeping the knife and cutting board sterile between different cuts. Sections were analyzed (2 to 3 replicates) for *E. coli* O157:H7 by plating on Tryptic Soy Agar with rifampicin (100 µg/ml). The purge generated following injection of each knuckle was also analyzed for the pathogen.

Results: The percentage gain in product weight following injection with water or brine solution was 3 to 10%. The purge released following injection with water or brine solution had *E. coli* O157:H7 levels of 6.2 log CFU/ml (scenario 1) and 3.1 to 3.6 log CFU/ml (scenario 2). *E. coli* O157:H7 was recovered from all sections of water- and brine-injected samples under both contamination scenarios, with similar counts obtained for the water and brine treatments. Higher counts of the pathogen were obtained in all sections under scenario 1 (1.9 to 3.6 log CFU/g) than scenario 2 (0.6 to 1.6 log CFU/g). Within each contamination scenario, similar counts were obtained between all sampled sections.

Poster

Significance: Moisture enhancement of beef via needle injection can transfer *E. coli* O157:H7 to the interior of whole muscle cuts when either the meat surface or brine solution is contaminated with the pathogen. The data may be useful in risk assessments for nonintact meat products.

P1-74 Transfer of *Escherichia coli* O157:H7 to Beef Steaks through Needle Tenderization

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Introduction: Cross contamination is one of the most important contributing factors in foodborne illnesses. Mechanical tenderization of meat may cause transfer of foodborne pathogens to subsequently tenderized product. However, there is limited information on cross-contamination during tenderization. Such products are classified as non-intact and if contaminated with *Escherichia coli* O157:H7 are considered adulterated.

Purpose: This study evaluated transfer of contamination of *E. coli* O157:H7 from surface-inoculated steaks to subsequently processed non-inoculated steaks through needle tenderization.

Methods: Beef steaks (7.5 × 6.25 × 3 cm; eye of round muscle) were surface-inoculated with 6 to 7 log CFU/cm^2 rifampicin-resistant *E. coli* O157:H7 (mixture of 8 strains). After inoculation, needle tenderization was performed using a hand-operated needle tenderizer (48 needles on a surface of 80 × 15 mm). After needle tenderization of each inoculated steak, six additional non-inoculated steaks were tenderized in sequence using the same tenderizer without sanitation. Steaks were tenderized one (single-pass tenderization), two (double-pass) or three (triple-pass) times. Samples were excised from the surface (0 to 0.1 cm depth) of each steak, and total bacterial (Tryptic Soy Agar, TSA) and *E. coli* O157:H7 (TSA with 100 mg/l rifampicin) populations were determined before and after tenderization. The study was replicated twice, with two samples each time.

Results: The results indicated that *E. coli* O157:H7 cells were transferred onto all non-inoculated steaks following needle tenderization. More specifically, the transfer of *E. coli* O157:H7 on the surface of non-inoculated steaks was 5.35 log CFU/cm^2 for the first cross-contaminated steak, 4.69 log CFU/cm^2 for the second, 4.65 log CFU/cm^2 for the third, 4.45 log CFU/cm^2 for the fourth, 3.91 log CFU/cm^2 for the fifth, and 3.81 log CFU/cm^2 for the sixth after single-pass tenderization. The transfer of *E. coli* O157:H7 following double-pass tenderization was 5.29 log CFU/cm^2 for the first cross-contaminated steak, 4.57 log CFU/cm^2 for the second, 4.45 log CFU/cm^2 for the third, 4.28 log CFU/cm^2 for the fourth, 3.79 log CFU/cm^2 for the fifth and 3.61 log CFU/cm^2 for the sixth. Similar trends in results were observed for triple-pass tenderization.

Significance: The study provided quantitative data for transfer of *E. coli* O157:H7 from a surface-inoculated steak to subsequent non-inoculated steaks and will be useful in risk assessments for non-intact beef products.

P1-75 Selection and Characterization of Cellulose Deficient Mutants of Shiga-toxin Producing *Escherichia coli* DSC

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Introduction: Shiga-toxin producing *Escherichia coli* (STEC) are known to have several defense mechanisms, one of which is the production of protective extracellular substances, such as cellulose.

Purpose: The goal of this study was to prepare pairs of STEC cultures useful for future research designed to address the role of cellulose in protecting the cells of STEC against environmental stress.

Methods: Spontaneous cellulose deficient mutants, 19D and 49D, were isolated, and the identities of the mutants and their respective parents, 19B and 49B, were confirmed. Growth characteristics of the STEC strains were determined using the phenotypic microarray (PM) technology.

Results: The two members within each STEC pair shared the same serotypes and similar PFGE profiles. Profound morphological differences were found, however, between the two types of cells. Strain 49B and 49D grew better than 19B and 19D in all three PM panels used in the study. The growth of 19B vs. 19D and 49B vs. 49D were significantly different only in the presence of two antibiotics on the antibiotic panel. On the osmolyte panel, 49D had significantly poorer growth than 49B only in broth supplemented with 4 different osmolytes. Strain 19D, however, grew similarly to 19B under these conditions. On the pH panel, significant differences in the growths of 19B and 19D were observed only in broth with pH 4.5 or 9.5 and supplemented with 3 to 4 different amino acids or trimethylamine-N-oxide. Strain 49B grew better than 49D only in broth with pH 9.5 and supplemented with five different amino acids. Additionally, 19B and 49B grew better than 19D and 49D, respectively, in broth supplemented with X-caprylate.

Significance: The two members of each STEC pair shared similar growth characteristics except under extreme stress. These strains could be useful in investigating the role of cellulose in protecting the cells of STEC against environmental stress.

P1-76 Tandem Repeat Stability in *Escherichia coli* O157:H7 is Dependent on the Duration and Type of Environmental Stress

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Introduction: Escherichia coli O157:H7 is an enteric pathogen that can survive in low numbers in water, in soil and on plants. Multilocus variable number tandem repeat analysis (MLVA) has become the method of choice for high-throughput subspecies typing of *E. coli* O157:H7. Comparisons of hundreds of isolates from environmental and clinical sources indicate considerable variation at 11 loci, with some loci apparently changing faster than others.

Purpose: The rate of mutation at these loci is of considerable importance to source tracking.

Methods: Mutation rates at these same loci have been measured by us and other research groups during repeated serial passaging on complete media. Since conditions in the environment are very different from those in the animal gut or

the laboratory, we have tested the rate of change of MLVA type under stressful conditions of low and high temperature, irradiation and long-term and short-term starvation.

Results: Under these conditions some loci (e.g. Vhec1) mutated readily (10^{-4}/generation or less), while others did not mutate at a measurable level. Repeated passage on complete media at 37, 25, 15 or 10°C showed that the mutation rate decreased as the temperature decreased. Likewise, increased temperature (43°C) caused a significant increase in mutation rate. Furthermore, exposure to UVC irradiation and sunlight increased the rate of mutation by increasing the size of insertions and deletions within the tandem repeats. However, MLVA type did not change in 28 strains re-isolated after survival for 3 months on plants. Likewise, under short-term starvation conditions in creek water for 7 days, the MLVA type also failed to change appreciably. Nevertheless, under long-term starvation conditions in which the bacteria are allowed to grow periodically, the mutation rate was elevated.

Significance: Therefore, more moderate, long-term environmental stresses foster alterations of MLVA type, but if the level or duration of the stress is such that the bacteria do not replicate, MLVA type appeared stable.

P1-77 Prevalence, Serotypes, and Virulence Genes of Shiga Toxin-producing *Escherichia coli* Isolated from Swiss Raw Milk Cheeses

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are among the most important causes of foodborne diseases. Public health problems associated with the consumption of unpasteurized milk and raw milk products are documented by recent foodborne infections. Traditionally, Swiss semi-hard and hard cheese is made from raw milk, with the natural microflora responsible for enhancing desirable flavor characteristics.

Purpose: The aim of this study was to assess the STEC prevalence in soft, semi-hard, and hard raw milk cheese within a three year monitoring program and to further characterize isolated strains.

Methods: The 1436 cheese samples were collected at the producer level within a national sampling plan during the period of March 2006 to November 2008. Each sample was enriched in brilliant green bile broth and screened by PCR for a region conserved between stx_1 and stx_2. STEC strains were isolated by colony dot-blot hybridization and further characterized by serotyping, typing of Shiga toxin genes, and detection of *eae* and *hlyA*.

Results: The proportion of cheese samples with *stx*-positive PCR results after enrichment was 3.7% in the year 2006 (n = 432), 6.3% in the year 2007 (n = 364), and 6.8% in the year 2008 (n = 640). All positive samples originated from soft and semi-hard raw milk cheeses. The 30 isolated STEC strains belonged to non-O157 serotypes, and stx_2 variants dominated among these strains. Production of stx_2 and stx_{2d2} subtypes is an indicator for severe outcome in infected patients. Three strains harbored *hlyA* (enterohemolysin), whereas none tested positive for the adhesion factor intimin (*eae*).

Significance: The results obtained reinforce the importance of soft and semi-hard raw milk cheeses as potential sources and vehicles for transmission of pathogenic STEC to humans.

P1-78 Efficacy of Ethanol as a Disinfectant for Inactivation of Human Noroviruses and Murine Norovirus

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Introduction: Human noroviruses (NoV) account for a large portion of the overall burden of foodborne disease. Studying the effects of disinfectants on NoV can lead to more effective ways of controlling the spread of illness.

Purpose: The purpose of this project is to compare commonly used disinfectants with respect to their efficacy for inactivation of several NoV as evaluated by a cell culture infectivity assay and/or quantitative reverse transcription PCR (RT-qPCR).

Methods: Ethanol (concentrations of 50%, 70% and 90% vol/vol) was evaluated against two representative genogroup II human NoV strains (GII.2 and GII.4) and the murine norovirus (MNV-1), a culturable surrogate. Experiments were performed in accordance with the *ASTM Standard Test Method for Efficacy of Antimicrobial Agents against Viruses in Suspension* (Designation: E 1052-96) using ~10^5 to 10^7 detectable units/test and a 30 s exposure period. Virus inactivation was quantified using RT-qPCR targeting the junction between the ORF1 and ORF2 (GII human NoV) and RNA polymerase region (MNV-1); infectivity assays were also performed for MNV-1.

Results: Log reductions of 0.3, 3.4 and > 3.6 were observed by infectivity assay (MNV-1) after 30 sec exposures to 50, 70 and 90% ethanol, respectively. By RT-qPCR, these numbers were 0.8, 2.8 and 2.2. For the human NoV GII.2 and GII.4 strains, there was no statistically significant ($P < 0.05$) reduction in titer control virus where compared to virus exposed to ethanol, regardless of concentration, as evaluated by RT-qPCR.

Significance: Although it appears that the human NoV is more resistant to ethanol than the MNV-1 surrogate, the absence of a cell culture model for human strains complicates conclusions. It is also possible that protection from stool accounts for the observed difference in ethanol sensitivity. Similar experiments are in progress using disinfectants with other proposed mechanisms of action.

P1-79 Characterization of the Transferability of Noviruses between Foods and Representative Surfaces

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Introduction: The human noroviruses (NoV) are responsible for a large proportion of foodborne disease, and poor personal hygiene practices of infected workers is the most common route of foodborne transmission. Their low infectious dose and environmental stability undoubtedly contribute to their success as agents of foodborne disease.

Purpose: The purpose of this study was to characterize the easy of transfer of representative NoV strains between foods and food contact surfaces, using a combination of quantitative real-time reverse transcription PCR (RT-qPCR) and cell culture infectivity assay.

Methods: Two model foods (lettuce and deli-sliced turkey), three representative surfaces [stainless steel, ceramic, and Formica®], and two viruses [Snow Mountain, and the cultivable surrogate murine norovirus (MNV-1)] were evaluated. Virus inoculum (1 10^{4-6} detectable virus units) was applied to sterile surface coupons, and transfer of virus to food was evaluated at various time-points (0, 15, 30, 60, 120 min) and pressures (100 and 1000 g/9 cm^2) by plaque assay and/or RT-qPCR, as appropriate.

Results: Statistically significant ($P < 0.05$) differences in transfer efficiency were observed over time (decreases) and with pressure (increases). For example, transfer of Snow Mountain virus (genogroup II) from ceramic to lettuce ranged from 0 to 9% (100 g/9 cm^2) and 0 to 26% (1000 g/9 cm^2); similar data were obtained for the other surfaces. When these experiments were repeated using MNV-1, transfer efficiency ranged from 2 to 8% and 1 to 11% at high and low pressures, respectively, using RT-qPCR; similar numbers were obtained by infectivity assay. Transfer efficiency also varied by food. Specifically, the transfer of MNV-1 from turkey to stainless steel ranged from 55 to 95% which was statistically significantly greater ($P < 0.05$) than transfer between stainless steel and lettuce under the same conditions.

Significance: We demonstrate that the human NoV and their surrogates are readily transferred from food preparation surfaces to foods, with the efficiency of such transfer affected by moisture, pressure, and food matrix.

P1-80 Hepatitis A Virus Survival during Low Heat Dehydration of Green Onion

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Introduction: Drying is one of the most common ways to preserve foods, including fruits and vegetables, and there is little information concerning the survival of Hepatitis A virus (HAV) during food dehydration processes.

Purpose: Because green onions have been linked to outbreaks of Hepatitis A virus (HAV), we studied the effect of dehydration on the survival of HAV on the surface of inoculated green onions. Dehydration effects were studied at two temperatures, 45°C and 60°C, which encompass the range of recommended drying temperatures for green onions.

Methods: Triplicate samples of green onions each were spot-inoculated with 10 µl HAV. The inoculated samples and uninoculated controls were placed in a home style dehydrator and dried at both 45° and 60°C for 20 h. Positive control samples were air-dried at ambient temperature for 30 min. HAV was eluted from the dried green onions using 3% beef extract. After filtration through a 0.45 µm membrane, different dilutions in duplicate aliquots were made. The dilutions were inoculated onto FRHK-4 cells grown in 6-well plates to assay for infectivity and quantify the number of viable HAV particles remaining.

Results: After dehydration of the green onions at 45°C, the level of reduction in the HAV population was estimated to be less than 1 log, compared to the initial level inoculated. At 60°C, approximately a 3-log reduction of HAV was observed.

Significance: More efficient inactivation of HAV during dehydration of green onions has been shown by the use of higher temperature.

P1-81 Capture of Human Norovirus Using Histo-blood Group Antigens (HBGA) as Binding Ligands

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Introduction: Human noroviruses (HuNoV) constitute an epidemiologically important group of foodborne pathogens. Because HuNoV are unculturable, methods for their detection in foods rely on virus concentration followed by molecular detection. Concentration methods are usually non-specific and may result in co-precipitation of PCR inhibitors.

Purpose: Based on recent findings that the HuNoV bind specifically to human histo-blood group antigens (HBGAs), we screened candidate HuNoV strains for such binding specificity. The ultimate purpose is to use these ligands to facilitate virus capture and detection from complex sample matrices.

Methods: Biotinylated synthetic HBGAs, including H type 1, H type 2, H type 3, Lewis a, Lewis b, and blood group type A were bound to streptavidin-coated magnetic beads. Viruses in serially-diluted fecal suspensions and in artificially contaminated foods were captured using the ligand-bound beads followed by detection using quantitative real-time PCR (RT-qPCR).

Results: Norwalk virus (representative genogroup I strain) bound specifically to H type 3 antigen. Binding efficiency and detection was impacted by sample matrix purity. For example, in highly purified (by ultracentrifugation) fecal suspensions, 12 to 30 genome copies could be detected by RT-qPCR, as compared to unpurified stool suspensions for which detection was 10-fold less sensitive. The Snow Mountain virus (genogroup II.2) also bound with high affinity to H type 3 antigen with a subsequent RT-qPCR detection limit of 200-300 genome copies. A fecal sample containing genogroup II.4 also bound to H type 3, with weak binding to Lewis b and moderate binding to H type 2, blood group type A, and Lewis a antigens; the detection limit for capture and detection of GII.4 was similar to that for Snow Mountain virus.

Significance: These results confirm the different binding patterns of different HBGAs for individual HuNoV genotypes. The approach shows promise for application to virus capture from complex sample matrices.

P1-82 Effect of Broad Spectrum Fertilizers on Human Picornaviruses
DSC

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Introduction: Contamination of ground waters by pathogenic microorganisms is common in many areas of the United States and public health concerns are increasingly focused on viruses. Enteric viruses are shed from their animal or human hosts into the environment and have the potential to survive, persist, and be transported by various routes to infect their new host. In agriculture, one means of transmission is water applied to crops through pesticide or fertilizer application.

Purpose: This assay was designed to determine the sensitivity of two environmentally-transmitted human picornaviruses, hepatitis A virus (HAV) and Aichi virus (AiV), to commonly used plant fertilizers. While HAV and AiV are similar in morphology, they differ in response to common inactivation measures.

Methods: Virus infectivity was first assessed with a traditional biocide testing method. Six broad spectrum fertilizers were evaluated (ammonium sulfate, ammonium nitrate, calcium nitrate, urea, Peters Excel, Triple Super Phosphate). Fertilizers were mixed according to manufacturer's instructions with sterile water. Viruses (10 µl) were dried (30 min) onto glass coverslips (7.2 log TCID50/ml AiV and 6.8 log TCID50/ml HAV), treated with the fertilizer (250 µl), and incubated for 10 min before neutralization with 750 µl of Letheen broth. Virus was recovered from the coverslips and infectivity assessed by TCID50. Control virus was assessed in each trial using water and Letheen broth only.

Results: Overall HAV was slightly more sensitive to the fertilizers, with reductions of > 1 log TCID50/ml for 5 out of 6 fertilizers. Calcium nitrite and urea showed the greatest reductions of 1.5 log TCID50/ml of HAV and AiV, respectively. Both ammonium sulfate and urea gave < 0.99 log TCID50/ml reduction of HAV. Peters Excel resulted in < 0.73 log TCID50/ml reduction of AiV.

Significance: The use of fertilizers may not inactivate viruses in contaminated water. Further assessment of virus in fertilizer preparations, in contaminated soil and on plants is under way.

P1-83 Norovirus: Foodborne or Pandemic Pathogen?
DSC

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Introduction: The Centers for Disease Control and Prevention (CDC) considers norovirus to cause the largest number of foodborne-related gastroenteritis cases in the United States. The association of norovirus with foodborne outbreaks through its collection tool focuses on the food worker as the typical source. Yet, many norovirus outbreaks are not foodborne in nature.

Purpose: This study examined the strength of association between food workers and food to norovirus in comparison to bacteria associated with foodborne-related gastroenteritis by whether norovirus had a direct (physical evidence), indirect (statistical evidence), or suspect (neither of the two) association with food or foodhandlers. The gap in the research is the evidence supporting the theory that norovirus transmission is the same as bacterial transmission.

Methods: This study determined the statistical association of outbreak related details from the electronic Foodborne Outbreak Reporting System (eFORS) between 1998 and 2006.

Results: The data showed norovirus were not associated with any specific foods related to outbreaks. It did show a high odds ratio of noroviral outbreaks indirectly related to food workers when compared with bacterial based (*Salmonella*-associated) outbreaks (OR = 15.84 ((CL 8.87 to 28.30), ($P < 0.01$)), but only a slightly similar one when directly related to food workers (OR = 1.41 (CL 1.11 to 1.78)) with *Salmonella* ($P < 0.01$). Yet the odds ratio of noroviral outbreaks directly or indirectly related to food, compared with *Salmonella*-associated outbreaks, was close to zero (OR = 0.40 ((CL 0.3117 to 0.5201), ($P < 0.05$) and OR = 0.28 (CL 0.2121 to 0.3648), ($P < 0.01$)), respectively). The data showed no similarity between proportion of the implicated and non-implicated numbers of outbreaks from norovirus and those from *Salmonella*. The analysis also showed a stronger similarity between proportions of food-handler implicated outbreaks from norovirus than from *Salmonella*.

Significance: An analysis showed, though, a significant emphasis was placed not on the food handler but on other indirect routes of transmision of norovirus in outbreaks. The analysis also indicated that norovirus transmission was not mainly through food. Norovirus transmission appeared to be person-to-person rather than through food and had more similarities to pandemic influenza than to transmission through gastroenteritis-associated bacteria. A change in surveillance of norovirus by health agencies could change food sanitation and general personal hygiene behaviors in person-to-person contact situations.

P1-84 Fate of Murine Norovirus-1 during Dairy Manure-based Composting
DSC

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Introduction: Norovirus is the leading cause of foodborne illness in the US, and recent studies have shown that norovirus can be shed by cattle and swine, with possible zoonotic transmission in the food chain. The application of manure on agricultural land could result in crop contamination. Composting is the common means of reducing pathogens in manure; however, little is known about the fate of norovirus during the composting processing, and such knowledge is important for agriculture practices.

Purpose: To determine the loss of infectivity of norovirus during the composting process.

Methods: One ml of 2×10^6 PFU/ml Murine norovirus-1 (MNV) was added to 2 g sterile solid dairy manure (DM), dried for 15 min and stored in a heat-sealed membrane bag (0.22 µm pore size). Composting was set up in a pile 0.3 m high and 1.2 m in diameter in an open field, using raw solid DM as material. The virus sample was placed in the center layer of the compost. Samples were collected at particular time points, at which times the temperature and water activity of the center layer of the compost were also measured. The sample was diluted with phosphate buffer and MNV infectivity was analyzed using the plaque assay.

Results: Compost temperature reached 61.7°C at day 1 and 65.2°C at day 2, after which it decreased to 47.8°C at day 5 and remained at ~45°C until day 15. The temperature then decreased to ~10°C at day 25 and was maintained at ~5°C at day 30. MNV was stable during the composting process. There were ~6.1 and 5.9 log PFU/ml infectious MNV at day 1 and day 2, respectively, and after 30 days, 5.3 log MNV were still detected. In contrast, Aichi virus had > 4 log loss of infectivity at day 1 and no viral RNA was detected at day 2.

Significance: The stability of MNV during the composting process results in a potential danger of eating fresh produce grown on land to which manure has been applied.

P1-85 DSC Comparison of Methods for Recovery of Hepatitis A Virus (HAV) from Fresh Produce and Detection of HAV by Real-time RT-PCR and Cell Culture

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Introduction: Hepatitis A virus (HAV) outbreaks associated with consumption of fresh fruits and raw vegetables have been reported in recent years in Korea. However, limited methods have been developed for detection of the virus in foods, and a method that isolates and detects HAV from fresh produces has been not standardized.

Purpose: The purpose of this study was to compare methods for elution and concentration of HAV on the surface of leafy vegetables and to develop a rapid, sensitive, and reliable detection method using real-time RT-PCR and cell culture.

Methods: Leafy vegetable samples (lettuce, Chinese cabbage) were artificially inoculated with HAV and processed by the sequential steps of homogenization, elution, concentration, RNA extraction, and real-time RT-PCR or cell culture, using FRhk-4 cell. Viruses were eluted from the food surface by two different elution buffers, buffer A [100 mM Tris-HCl, 50mM glycine, 3% beef extract pH 9.5] and buffer B [250 mM Threonine, 300 mM NaCl, pH 9.5]. In addition, an ultrafiltration method was evaluated by comparing it with a widely used viral concentration method, PEG precipitation. The FRhk-4 cells were infected with viruses recovered from the samples to determine infectivity of the viruses, and integrated cell culture real-time RT-PCR was performed at 72, 120,168, 216, 264, and 288 h post infection.

Results: The sequential step of elution using buffer A and ultrafiltration were evaluated as a primary virus concentration step for vegetable samples, and detection limit of this method was found to be 1.9×10^2 RNA copies/g. Cytopathic effect and rapid decrease of Ct value were observed at 14 days post infection in the FRhk-4 cell infected with the viruses extracted and concentrated by buffer A and ultrafiltration.

Significance: The optimized detection method evaluated in this study was successful in detecting HAV contamination from a wide range of fresh produce, by molecular and cell culture methods. This method could be used for studies and routine surveillance of viral quality of vegetables.

P1-86 Prevalence of *Vibrio cholerae, V. parahaemolyticus* and *V. vulnificus* in Retail Frozen Shrimp Determined with a Real-time PCR Assay

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Introduction: Vibrio species are a frequent cause of bacterial seafoodborne disease. The prevalence of *Vibrio* in coastal waters and in aquaculture operations, combined with globalization of food production, may place consumers at risk of seafoodborne diseases.

Purpose: The study was initiated to assess the prevalence and levels of *Vibrio cholerae* (Vc), *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) in retail frozen raw and cooked shrimp, using a multiplex real-time PCR assay specific for these *Vibrio* species.

Methods: Twenty-five grams of thawed whole shrimp were homogenized 1:10 in alkaline peptone water. After 18 h incubation at 35°C, enrichments were streaked on TCBS, mCPC and Vibrio CHROMagar™ plates, and samples were processed for the BAX® system *Vibrio* assay. Presumptive colonies were propagated for cultural confirmation. Initial contamination levels were determined by the FDA-BAM MPN method for Vp and Vv.

Results: PCR screening of imported frozen raw and cooked shrimp demonstrated 64% (n = 14) of retail batches contaminated with culturable Vp. Of these, 50% of the batches of raw shrimp were also contaminated with viable Vc or Vv. Cycle threshold (Ct) values indicated Vp levels were 2 logs higher than Vc or Vv after enrichment. MPN analysis revealed an initial 9300 MPN/gm for Vp and 6.5 MPN/gm for Vc for one retail batch of raw shrimp. For Vp positive raw shrimp batches, 100% of individual shrimp tested at 1:10 enrichment were positive for Vp. In contrast, sporadic Vc contamination (6 confirmed positive out of seventy 25 g samples tested) was observed in one retail batch. Morphologic and molecular characterization by ribotyping revealed the presence of multiple strains of *Vibrio* in dually contaminated samples.

Significance: The prevalence of Vp and other *Vibrio* species in retail shrimp revealed in this study reinforces the need for both safe seafood preparation practices and increased microbial quality standards.

P1-87 Toxin Genes Characterization and Antibiotic Susceptibility Patterns of Emetic-type *Bacillus cereus* Korean Isolates

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Introduction: Many studies of diarrheal toxin-producing *Bacillus cereus* have been reported, but little study has been done on the emetic toxin-producing *B. cereus* in Korea. We first reported the identification of emetic toxin-producing *B. cereus* among *B. cereus* strains isolated from grains, Korean rice cake, water, and human outbreak feces. Many investigators reported that emetic toxin-producing *B. cereus* produced diarrheal toxin.

Purpose: The objective of this study was to determine the diarrheal toxin genes such as nhe, hbl, and cytK and to analyze the antibiotic susceptibility patterns of emetic type *B. cereus* Korean isolates.

Methods: PCR amplification was performed to detect the *B. cereus* diarrheal toxin genes such as hbl A, hbl B, hbl C, hbl D, nhe A, nhe B, nhe C, and cytK. The production of enterotoxin by the emetic toxin-producing *B. cereus* isolates was determined using a reversed-passive latex agglutination kit (BCET-RPLA). The antibiotic susceptibility patterns of the isolates were determined by the disk diffusion susceptibility test.

Results: Three emetic toxin-producing *B. cereus* isolates (*B. cereus* KUGH 27, 85, 164) showed 755 bp band, 743 bp band, and 683 bp band amplified from nhe A, nhe B, and nhe C gene, respectively. The hbl and cytK genes were not detected. The antibiotic susceptibility patterns of all emetic toxin-producing *B. cereus* isolates showed resistance to ampicillin, cefepim, oxacillin, and penicillin.

Significance: This study revealed that three emetic toxin-producing *B. cereus* isolates tested in this study showed the diarrheal toxin gene (nhe). Thus, diarrheal and emetic toxin should be constantly monitored to prevent misdiagnosis between diarrheal and emetic type of food poisoning.

P1-88 Identification of Emetic Toxin (Cereulide) Producing *Bacillus cereus* Isolated from Human Outbreaks and Food in Korea

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Introduction: Bacillus cereus, a gram-positive spore-forming bacterium, is isolated from a wide range of foods and environments. *B. cereus* can cause diarrheal and emetic types of food poisoning. The emetic type has been reported to be predominant in the UK and Japan, and Korean diets are very similar to those of Japan. However, the emetic type has not yet been reported in Korea.

Purpose: The purpose of this study was to determine the emetic toxin (cereulide) producing *B. cereus* among *B. cereus* strains isolated from human outbreak feces and foods in Korea, using PCR and HPLC-MS methods.

Methods: Amplification of the non-ribosomal peptide synthetase (NRPS) gene from the isolates was conducted with two primer pairs (CER1 and EMT1) to select the putative emetic toxin (cereulide) producing *B. cereus*. HPLC-MS analysis was performed and the emetic toxin (cereulide) was detected using ion trap mass analyzer to confirm the emetic toxin (cereulide) producing *B. cereus*. *B. cereus* F4810/72 was used as the reference strain.

Results: In total, 112 of *B. cereus* strains isolated from grains (n = 43), Korean rice cake (n = 8), side dishes (n = 7), water (n = 4), and human feces (n = 50) were analyzed. Four of 112 *B. cereus*. strains were detected using the CER1 and EMT1 primer pairs (188bp band). The HPLC-MS spectrum showed that three of four putative emetic toxin (cereulide) *B. cereus* exhibited ion ranges (m/z) of 1,170 (NH_4^+ adduct) showing the same mass spectrum as the reference strain (*B. cereus* F4810/72).

Significance: The results indicated that the PCR method for identification of emetic toxin (cereulide) producing *B. cereus* should be confirmed using other methods, such as HPLC-MS, the sperm motility assay, and the HEp-2 cell vacuolation assay.

P1-89 Prevalence of MRSA in Food Samples Associated with Foodborne Illness

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Introduction: Staphylococcus aureus is an important human pathogen associated with food poisoning known to cause symptoms such as nausea, vomiting, diarrhea and abdominal pain. The emergence of methicillin-resistant *S. aureus* (MRSA) is a continuing problem for public health; in Alberta, community-acquired MRSA infections unrelated to food are on the rise. The extent of MRSA associated with foodborne illness is not known.

Purpose: The Alberta Provincial Laboratory for Public Health (APLPH) provides microbiological testing for all food samples submitted as a part of a foodborne investigation. This study determined the prevalence of methicillin resistance among *S. aureus* isolates detected in food samples.

Methods: Between 2007 and 2008, a total of 290 food samples associated with food illness investigations submitted to the APLPH were cultured for *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Aeromonas*, *Campylobacter*, *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, and *Yersinia*. All *S. aureus* isolated were screened for methicillin resistance using disc diffusion (30 ug Cefoxitin; 1ug Oxacillin) and confirmed by a PBP2 latex agglutination kit (Denka Seiken, Japan) as per the Clinical and Laboratory Standards Institute guidelines.

Results: S. aureus was isolated from 37 of 290 (12.8%) of food samples submitted to the APLPH. Of the *S. aureus* isolated from food, a total of 2.7% (1/37) were found to be MRSA.

Poster

Significance: Incidence of MRSA among *S. aureus* originating from cases of foodborne illness are low despite the emergence of community acquired MRSA infections seen in Alberta.

P1-90 Efficacy of Ovotransferrin against Pathogenic and Spoilage Organisms in Laboratory Broth Medium and a Model Milk System

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Introduction: Milk is a highly perishable food product that needs adequate refrigeration to delay microbial spoilage and growth of pathogens. In some areas of developing countries there is a lack of refrigeration at the milking parlor and in transport trucks. In this regard there is a crucial need for a natural antimicrobial intervention to suppress microbial growth in raw milk and maintain wholesomeness of this product during transport to the milk pasteurization plant.

Purpose: The purpose of this study was to evaluate the efficacy of ovotransferrin (OTF), a natural antimicrobial derived from the hen egg whites, against both pathogenic and spoilage organisms in laboratory broth and a model milk system at 25°C.

Methods: Brain Heart Infusion broth (BHI) was prepared with 100 mM sodium bicarbonate and 0 (control), 20 and 60 mg/ml OTF. OTF at 0 and 60 mg/ml was used in UHT milk. BHI and UHT milk were inoculated with *Escherichia coli* 0157:H7, *Listeria monocytogenes, Salmonella*, or *Pseudomonas fluorescens* at 10^4 CFU/ml. All inoculated samples were held at 25°C and sampled every 3 h for 18 h. Bacterial growth was monitored spectrophotometrically (OD 600nm) and by plate counts for BHI and UHT milk, respectively.

Results: In BHI pathogens in control (0 mg/ml) grew after 9 h. OTF at both 20 and 60 mg/ml increased the lag phase of all the pathogens and decreased growth rate over 18 hr. OTF (60 mg/ml) exhibited a greater growth inhibitory effect than 20 mg/ml OTF. Compared to the control, differences in optical density caused by 60 mg/ml OTF were 0.10, 0.24, 0.82, and 0.24 for *L. monocytogenes*, S*almonella*, *E. coli*, and *P. fluorescens*, respectively, at 12 h. *E. coli* was the most sensitive pathogen, exhibiting no increase in optical density through the 18 hour period. Generally, no significant inhibition of the organisms occurred in UHT milk with OTF at 60 mg/ml.

Significance: Based on broth studies, OTF has good potential for controlling growth of foodborne pathogens at 25°C. The observed lack of bacterial growth suppression by OTF in UHT milk warrants further research on ways to enhance the antibacterial effect of OTF in milk.

P1-91 Prevalence of *Clostridium difficile* within Ontario Pig Farms—The Foodborne Link

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Introduction: Clostridium difficile infection (CDI) is the most common cause of nosocomial and antimicrobial-associated diarrhea in North America, accounting for over 25,000 deaths in recent years. The pathogen has been commonly linked to hospital-acquired infections whereby disruption of the gastro-intestinal tract microflora via administration of antibiotics enables *Cl. difficile* to proliferate and produce toxins. BI/NAP1/027 is a hypervirulent strain that has been associated with outbreaks internationally and in surveys performed in 2000-2004, has been isolated in animal production facilities (notably pigs and cattle), in addition to retail meats. In the last two years, there has been a sharp increase in community acquired infections in which the affected people have no immediate history of antibiotic usage or contact with health care environments. Interestingly, *Cl. difficile* ribotype 078 is increasingly implicated in community acquired infections, suggesting that the source is different from that of ribotype 027.

Purpose: The following study, supported by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), has undertaken a survey to determine the prevalence of *Cl. difficile* within Ontario pig farms. Of interest was to determine the prevalence of the pathogen, distribution of ribotypes and toxin production by the recovered isolates.

Methods: Fecal swab and manure effluent lagoon samples were screened for the presence of *Cl. difficile*. Pro-disk was applied for confirmatory testing, with ribotyping and toxinotyping being performed using PCR.

Results: Fecal and effluent samples (133) were collected from 52 farms located within Southern Ontario. *Cl. difficile* was recovered from 15 farms (28% prevalence). The majority of isolates recovered belonged to ribotype 078 (16/20 isolates), with ribotype 027 (commonly linked to health care acquired infections) being less prevalent (1/20). All the isolates produced toxin A & B in addition to binary toxin, confirming potential pathogenicity towards neonatal pigs and humans.

Significance: The prevalence of *Cl. difficile* is high within Ontario pig herds and a shift from the dominance of ribotype 027 to the strain implicated in community acquired CDI (ribotype 078) has occurred, providing further evidence of a foodborne link.

P1-92 Development of Random Genomic DNA Microarray Chip for the Detection of Foodborne Pathogens

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Introduction: Consumer concerns regarding their health care have increased, resulting in greater demand for safer foods. Foodborne pathogens have ranked as one of the most serious food risks. To develop effective strategies for producing foods free of foodborne pathogens, rapid and simultaneous detection methods for pathogenic bacteria in food products are essential.

Purpose: This study was performed to develop a DNA chip to detect foodborne pathogens such as *Listeria monocytogenes*, *Enterobacter sakazakii*, and *Yersinia enterocolitica* from various types of foods rapidly and simultaneously.

Methods: Genomic DNA of foodborne pathogens (*L. monocytogenes*, *E. sakazakii*, *Y. enterocolitica*) was fractionated by use of several pairs of restriction enzymes. After size fractionation of the genomic DNA fragments, random genomic libraries for each bacterium were constructed. Randomly selected DNA fragments were amplified by PCR and the DNA fragments were affixed to a slide glass to fabricate the DNA microarray chip. After the DNA chip was prepared, its ability to detect *L. monocytogenes*, *E. sakazakii*, and *Y. enterocolitica* simultaneously from laboratory media was confirmed.

Results: We were able to construct a chip that contains more than 50 random genomic DNA fragments of *L. monocytogenes*, *E. sakazakii*, and *Y. enterocolitica*. The DNA microarray chip was tested to confirm its ability to detect specific bacteria in laboratory medium. We were able to detect *Listeria monocytogenes*, *Enterobacter sakazakii*, and *Yersinia enterocolitica* simultaneously through the fluorescent signal by cross-hybridization within 24 h. The DNA microarray chip was able to clearly distinguish those microorganisms without any interference.

Significance: These results suggest that the DNA microarray chip will be useful in detecting specific bacteria from various types of food products rapidly and simultaneously without requiring any genus level sequence information from pure isolates.

P1-93 Microbiological Safety during Cold Delivery of Food Ingredients Supplied to Elementary School Food Services in Korea

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Introduction: Microbiological counts on food ingredients after processing are increasing in school kitchens, and distribution systems are not clean. In 2003, HACCP programs were established in school kitchens, but outbreaks in schools have not decreased.

Purpose: The purpose of this study was to analyze the distribution systems and evaluate the need for changes to increase the microbiological safety of food ingredients (Ready-to-Eat vegetables, soybean sprouts, process food) supplied to elementary school food services.

Methods: Ten food ingredients and 13 factories in the provinces of Daegu and Gyeongbuk were chosen. The samples were collected at each stage of delivery from factory to school. Total plate count (TPC), coliform groups, *Escherichia coli*, *Salmonella* species, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus* were tested.

Results: Food ingredients (fish, chicken, non processed products, frozen food) were not separated from each other in the refrigerator vans during the stage of direct delivery to schools (-10°C). After the ingredients arrived at the school, total plate count and coliforms were detected, as follows: peeled bellflower roots (10^6, 10^5 CFU/g), peeled onion (10^4, 10^1 CFU/g), boiled bracken (10^3 to 10^5, 10^1 to 10^4 CFU/g), seaweed 10^3, 10^3 CFU/g), soybean sprouts (10^5 to 10^7, 10^3 to 10^6 CFU/g), soybean curd (< 5 to 10^3, < 5 to 10^5 CFU/g), starch jelly (< 5 to 10^3, < 5 CFU/g). *E. coli*, *S. aureus*, and *B. cereus* were detected (10^1, 10^2, 10^2 CFU/g) on peeled bellflower roots; *S. aureus*, *L. monocytogenes*, and *B. cereus* were detected (10^2, 10^1, 10^1 CFU/g) on soybean sprouts.

Significance: Distribution systems must have fewer stages of delivery, monitoring systems of the distribution companies need to be stricter, and food ingredients need to be sanitized during processing so that school kitchens do not have the sole responsibility for the safety of the food served to school children. A more coordinated effort must be made to integrate the distribution systems into the school kitchen HACCP programs.

P1-94 Organizational Factors Influencing Employees to Follow Food Safety Practices

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Introduction: Calls to take a multidisciplinary approach to food safety research and consider the behavioral or socio-psychological aspects related to adopting food safety practices have been made for more than 20 years. Recent studies focusing on individuals identified several factors that are predictors of food safety behavior in commercial settings. Workplace factors also play a role in whether or not individual employees follow food safety practices.

Purpose: The purpose of this study was to identify the organizational factors that influence production workers in Ontario meat plants to follow practices required by the establishments' food safety management systems.

Methods: In depth semi-structured interviews with 27 owners, senior managers, food safety personnel and/or production employees at nine establishments were triangulated with reviews of food safety management system records. Semi-structured observations were conducted in five of the establishments. Additionally, three focus group interviews were held with food safety consultants, provincial meat plant auditors and federal meat plant auditors. The 326 pages of verbatim transcriptions from the interviews, as well as field notes, were analyzed using NVivo 8 software.

Results: Focus group participants unanimously identified management commitment as the primary factor influencing the implementation of food safety management systems in meat plants. Management personnel indicated they had roles in supporting the food safety management systems such as providing resources (both financial and human), employee supervision and correction, and ensuring that they follow food safety related protocols themselves. Data from food safety personnel and production workers supported some management perspectives. Not all senior managers/owners appeared aware that their behaviors could negatively influence employee food safety practices. A cooperative work environment was linked to establishments where workers who took inappropriate actions accepted correction from food safety personnel and/or peers as well as supervisors.

Significance: The qualitative data describe organizational factors that influence individuals in following food safety management system protocols in Ontario meat plants. Confirmation of the key factors that influence program implementation among employees may assist in targeting interventions at the management or organizational level.

P1-95 Handwashing Behavior in Foodservice: Development of a Research Instrument

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Introduction: Proper handwashing is identified as one of the most effective ways to prevent foodborne illnesses. However, research shows that compliance with acceptable handwashing practices by foodservice workers was low. Better understanding of workers' handwashing behaviors can lead to development of effective training programs to improve compliance.

Purpose: To adapt measurement scales of determinant constructs based on the Theory of Planned Behavior to foodservice workers' handwashing behavior; to construct and pretest a questionnaire using the revised measurement scales that can be used in future studies examining handwashing behavior in foodservice establishments.

Methods: A self-administered questionnaire used for healthcare workers was adapted to foodservice workers. A discussion group was held to identify additional items. The resulting questionnaire consisted of 5 sections. Section 1 measured semantic differentials measuring attitudes towards handwashing. Section two contained 54 Likert-scale statements measuring (a) subjective norm, (b) behavioral belief strength, (c) intention, (d) behavioral belief strength, (e) normative belief strength, (f) control belief strength and (g) subjective knowledge. Section three contained eight items measuring handwashing outcome evaluation. Section four measured self-reported behavior. Section five was demographics. Questionnaires (n = 250) were distributed at four participating universities across the United States.

Results: There were 144 usable questionnaires. Half of the sample had worked in foodservice for 1 to 5 years, with the majority having received some type of food safety training. There were strong single factor loadings of items for each factor, except attitude. Thus, one attitude item was removed. Cronbach Alphas ranged from .76 to .95, suggesting high internal consistency, except .61 for perceived behavioral control. To address the issue of reliability, an item was added to perceived behavioral control. The final questionnaire contained 88 items measuring seven determinant constructs to handwashing behavior as well as self-reported handwashing, and demographics.

Significance: The questionnaire that was developed will be useful for future studies in measuring factors that influence handwashing behavior of foodservice employees. This will allow foodservice establishments to: better train employees on handwashing, understand how employees view handwashing, and improve handwashing compliance in foodservice establishments. This could potentially help to prevent cases of foodborne illnesses that resulted from improper handling of food, cross contamination, and poor personal hygiene.

P1-96 Implementation and Efficacy of Self Audits, Community Engagement and Food Safety Employee Training in Minneapolis Food Service Establishments

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Introduction: The restaurant industry employs 13.1 million people, and 59% of reported foodborne illness outbreaks were associated with restaurants in 2005. Outbreaks are usually related directly to food-handler error. Many food service establishments do not understand the significance of a regulatory inspection and how they can utilize these inspections in reducing the risk of a foodborne outbreak. In addition, Certified Food Managers or the Person-in-Charge lack the materials to provide necessary food safety training for front line employees.

Purpose: Implementation of a self-audit program, food safety employee training and community engagement to meet state and local regulatory food codes as well as establish systems of food safety and security measures to protect against contamination of food and potential foodborne outbreaks.

Methods: A self-audit was developed based on the Minnesota Food Code and translated into nine languages. The City of Minneapolis conducts an estimated 3,000 risk-based food inspections per year. Establishments that had high numbers or repeated critical violations were approached to voluntarily participate in the self-audit program, and attend monthly community engagement sessions addressing topics such as personal hygiene, cleaning/sanitation, cross-contamination, time/temperature and food security management. In addition, Food Safety Employee Training based on the self-audit was conducted on-site, either in English, Spanish or Somali.

Results: Inspection reports of 123 food establishments were compared before and after introduction to the self-audit program and food safety employee training. Of the restaurants participating in the self audit program and training sessions, a 40% decrease ($P = 0.0002$) in critical violations was observed. Participants also demonstrated statistically significant improvement in employee health and hygiene ($P = 0.002$), protection from cross contamination ($P = 0.002$), time and temperature logs ($P = 0.001$), use of chemicals ($P = 0.013$) and food security management ($P = 0.0006$).

Significance: The evaluation and data collected appears to demonstrate that the implementation of a monthly self-audit program as well as food safety employee training led to uniform and comprehensive food protection, a reduction in critical violations and ultimately a reduction in the number of foodborne illness investigations.

P1-97 Assessing the Training Resource Needs of Retail and Food Service Professionals

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Introduction: The US retail-foodservice industry includes over one million establishments. Food safety, a daily concern in these operations, is commonly addressed through training. An effective food safety training program is critical to keeping foods safe. Unfortunately, most food safety professionals are facing increasing challenges due to shrinking budgets, higher work loads, and limited time to prepare for and conduct training.

Purpose: The purpose of this project was to identify the specific challenges food safety professionals face when training retail and foodservice workers and to identify those resources that professionals need to more effectively conduct food safety training.

Methods: Six focus group sessions were held in 2008, at the annual National Environmental Health Association (NEHA) meeting in Tucson, AZ. Thirty participants attended one of six 50-minute sessions conducted by a trained interviewer. Participants were NEHA conference attendees, and nearly all stated that food safety training and education was one of their job responsibilities. A series of semi-structured questions related to food safety in the retail food environment were asked of all groups. Responses were audiotaped and then transcribed. The focus group protocol was approved by the Clemson University Internal Review Board.

Results: A general consensus of focus group participants was that food safety at the retail-foodservice level needs improvement. Specific challenges to training identified were: low literacy levels, language, time, money, interest, skills, and motivation. While thousands of food safety training resources are currently available, most session participants stated that more high-quality, science-based resources that could be modified to meet local conditions were needed. Participants also indicated that an on-line, easy-to-search repository was needed to minimize duplication of material development and to decrease the time needed to locate materials.

Significance: The findings from this study are being used to guide Retail and Foodservice Food Safety Consortium (RFSC) work on the development of a collection and review protocol for food safety resources. This information is also being used in the development of a national Web site that will contain many of these resources.

P1-98 Outreach Program to Provide Food Safety Education to Volunteer Food Handlers at Large Church-related Community Food Events

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Introduction: Churches are treated differently from commercial food establishments under Minnesota statutes and typically do not receive oversight from environmental health agencies. Churches routinely host meals for their congregations; however, many also periodically host large community events which are advertised to the public and require paid admission. Food preparation for these events is typically performed by volunteers not trained in food safety for large events.

Purpose: To describe foodborne disease outbreaks associated with church-related community events in Minnesota from 2004 to 2008 and to describe an outreach project to provide food safety training to volunteer food handlers at community events.

Methods: We reviewed church-related foodborne outbreaks in Minnesota from 2004 to 2008. We created an outreach program to provide food safety training to food preparation volunteers at church-related community events. Pre-session and post-session evaluations administered at each class determined participants' perception of their increase in food safety knowledge on a scale of 1 to 4.

Results: Four foodborne disease outbreaks associated with church-related community events occurred in Minnesota from 2004 to 2008. The median number of documented cases per outbreak was 20.5 (range, 12 to 46). Two outbreaks were caused by *E. coli* O157:H7, one by *C. perfringens*, and one was suspected to be caused by *C. perfringens*. The *E. coli* O157:H7 outbreaks resulted in three cases of hemolytic uremic syndrome and one death. Contributing factors to these outbreaks included cross-contamination, temperature abuse, and an unapproved food source. Eight classes (with 159 people) were conducted as part of the outreach project. Participants increased their scores on all seven measures of knowledge and ability (from a mean of 2.7 to 3.7).

Significance: Large church-related community food events have led to multiple foodborne outbreaks in Minnesota. It is unclear whether statewide regulation of these events would be feasible and effective. A voluntary education program has potential to provide critical food safety information to volunteers involved in these events.

P1-99 Understanding Food Safety Situation Pertaining to Asian and Hispanic Restaurants

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Introduction: Ethnic foods have gained popularity in the United States in recent years, and one of the most important outlets for these foods is ethnic food restaurants. Asian and Hispanic restaurants are among the most popular. Based on CDC data from 1990 through 2000, these cuisines face different food safety challenges.

Purpose: To better understand food safety needs for Asian and Hispanic restaurants, we surveyed ethnic food restaurants in California and Florida, two states with high numbers of ethnic restaurants.

Methods: Anonymous surveys containing 45-item questionnaires were mailed to 1,200 randomly selected Asian and Hispanic restaurants from each state 3 times over a period of 3 months. The surveys did not collect data that would specifically identify individual restaurants.

Results: The overall response rate was 18%, with a higher rate (24%) in Florida than in California (12%). This is considered a high response rate for this type of survey. The majority of respondents (74%) in both states were from Asian restaurants (mainly Chinese), followed by Hispanic restaurants (24%) (mainly Mexican). The remainder (2%) did not specify operation type. The majority of respondents (36%) employed more than 10 people, with 28% employing 3 to 5 people, 26% employing 6 to 10 people, and 8% employing 1 to 2 people. The majority of employees were reported to be between the ages of 22 and 64. The majority of the respondents (74%) expressed food safety concerns, citing language barriers, time, and cost as obstacles to meeting their food safety goals. Respondents reported using ServSafe® (69%), FSP Certification (17%), and Thomson Prometric (3.5%) as food safety certification programs.

Significance: This study documented specific food safety training needs of Asian and Hispanic restaurants, and it will be the first step toward customizing food safety training for these types of establishments.

P1-100 Food Safety Training Priorities for Evacuation Shelters Operated by Faith-based Organizations: An Expert Survey Using Discrete Selections

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Introduction: In large disaster situations like hurricanes, faith-based organizations offer food and shelter for evacuees. Previous studies found organizers of these evacuation shelters lacked food safety knowledge and failed to provide adequate food safety training before opening shelters. Because these shelters are operated with non-professionals such as volunteers and even evacuees, training must be provided to ensure food safety.

Purpose: The purpose of this expert survey was to identify contents and priorities of food safety training for evacuation shelters operated by faith-based organizations where no organized assistance was provided by Red Cross or Salvation Army.

Methods: One hundred food safety experts in the US were randomly selected from a directory of about 200 food safety researchers available on a USDA Web site. Seventeen unique food safety behaviors in five categories were repeatedly paired to form 45 discrete selection questions. From each pair, participants chose one behavior they felt more important than the other for food safety and rated its relative importance using a 3-point scale. A utility score measuring relative importance of each variable was created and analyzed using paired-samples *t*-tests and repeated measures MANOVA to identify priorities ($P < 0.05$).

Results: Sixty-two experts with an average of 15.4 years of food safety research experience provided usable data. Out of five categories, avoiding cross-contamination was regarded as most important (1.5 ± 0.5) followed by personal hygiene (1.4 ± 0.6), keeping food at safe temperatures (1.0 ± 0.3), and adequate cooking (0.8 ± 0.3). Among individual behaviors, washing hands (1.9 ± 0.5) and cutting utensils (1.5 ± 0.6) properly after handling raw meat/chicken were considered as most important. Using a thermometer to ensure complete cooking of meat/poultry (1.4 ± 0.5), not eating raw seafood (1.2 ± 0.8), and washing hands before food handling (1.2 ± 0.5) were also considered as important. However, not using home canned food, keeping cooked food hot during service, and thawing frozen meat in the refrigerator were not viewed as important (0.4 ± 0.6, 0.5 ± 0.4, & 0.7 ± 0.3, respectively) by experts, compared to other food handling behaviors in evacuation shelters.

Significance: The data suggest that food safety training in evacuation shelters, where training time is limited, should focus on preventing cross contamination, personal hygiene, and time and temperature control. A future study identifying differences in perceived importance of food handling behaviors between food safety experts and evacuation shelter operators/volunteers may identify gaps in risk perceptions and provide further training priorities.

P1-101 A New Internet Training Course on Current Good Manufacturing Practices (GMPs)

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Introduction: A new Internet training course that summarizes the requirements of the current Good Manufacturing Practices (21 CFR Part 110) is now available.

Purpose: The course consists of 12 Modules that cover all of the requirements in FDA's current GMP regulation and provides practical suggestions for industry to help their facilities meet these requirements. This Internet course is designed to train middle level managers, supervisors, quality control personnel and others who have responsibility for compliance with current GMPs in food processing, wholesale and warehouse operations.

Methods: The course was designed by a team of food safety professionals using feedback from a national survey of the food industry conducted in 2006. This project was funded through a grant from the National Integrated Food Safety Initiative (Grant No. 05-51110-03291) of the Cooperative State Research, Education and Extension Service, US Dept. of Agriculture.

Results: The GMP Internet course consists of 12 Modules that cover all of the requirements in FDA's current GMP regulation and provides practical suggestions for industry to help their facilities meet these requirements. Online registration and background information is available at http://gmptraining.aem.cornell.edu The course is hosted and managed by Cornell University and New York Sea Grant. Course features include examples of good and bad practices on "GMP TV", downloadable PDF files of the course content, checklists designed to help firms develop operating and sanitation procedures, and extensive links to additional resources. Students have the option of viewing course materials "live" on the Internet, downloading content to their computer, or listening to audio files of each page. A Spanish language version is also available. Individuals who complete the course will receive a Certificate of Course Completion, and have access to five In-Plant Training Modules that can be used for additional on-site training of line and production workers on critical components of the GMPs, such as hand washing, personal hygiene, cleaning and sanitizing, and process controls.

Significance: Evaluation feedback from several hundred individuals who have completed the course has been received. Over 90% rated the course excellent or very good, and indicated that the material is easy to read and understand. Almost all respondants indicated that they would recommend the course to others. This feedback provides evidence that this new Internet resource can provide an effective training tool for employees responsible for compliance with GMPs for food processing, storage and distribution firms that handle many different commodities.

P1-102 Impact of Education on Food Selection, Storage and Handling Practices of Rural Families

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Introduction: Rural families in close contact with farm animals may be at higher risk for contracting zoonotic diseases, including listeriosis, than families without such contact.

Purpose: As part of a large multi-state study, we examined the impact of food safety education with rural farm families on home environmental and food consumption/handling practices associated with increased risk of foodborne illness, particularly listeriosis.

Methods: Rural farm families with children in Northern Colorado were recruited to participate in a 3-year study. In phase one, researchers collected background questionnaires, then conducted home audits to assess overall kitchen and refrigerator cleanliness and document unsafe food storage or handling practices. The household's primary food preparer was then interviewed about food preparation, storage, cleaning and consumption practices. Following data collection, participants were provided with refrigerator thermometers and foodborne illness prevention materials, including home cleaning/sanitizing guidelines. In phase two, subjects participated in a one-hour web-based food safety education program and received additional educational materials. In phase three, data collection methods used in phase one were repeated with each household, along with a post-project survey.

Results: Of the 54 families recruited, 52 completed the 3-year study. Knowledge and awareness of *Listeria monocytogenes* increased significantly ($P < 0.001$), with 5.6% indicating high awareness pre vs. 68.3% post study. Two-thirds of participants indicated they had made changes in food selection, handling or storage practices as a result of study participation. Among participants reporting changes, more frequent hand washing (60%), increased monitoring of "use-by" dates (57%) and refrigerator temperature (46%), more frequent cleaning and sanitizing of kitchen sinks and food preparation surfaces (40%) and more rapid turnover of deli meats (40%) were the most frequently mentioned changes made. On a 5-point scale of 1 = very unclean to 5 = very clean, observed cleanliness of participant refrigerators increased from 3.1 pre to 4.1 post. Mean refrigerator temperature was 38.4°F pre and 37.3°F post.

Significance: Results suggest that education specific to factors associated with risk of listeriosis may help promote safer practices among rural farm families.

P1-103 Content Development for an Educational Workshop on Pre-harvest Food Safety Targeting Beef Feedlot Managers

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Introduction: Feedyard managers may not have accurate knowledge of current issues concerning pre-harvest food safety. Training feedyard managers on methods of pathogen control could improve the safety of the beef supply.

Purpose: The purpose of this study was to determine training needs for feedyard management through survey-based research.

Methods: In the first stage of data collection, we determined the most important topics to be presented at a pre-harvest food safety workshop directed to feedyard management. Industry experts as well as academia experts in the area of food safety were the source of the topics. Instrumentation was single item open-ended questions utilizing the Delphi method. After the initial topics were gathered, experts were then asked to specify their level of agreement of which topics should be taught. Following this stage, the experts were then asked to review items they chose to include and to determine if there were any items they would like to reconsider and not include in the workshop. The final stage of data collection consisted of ranking topics on level of importance. Those items with the highest level of importance were kept as curricula topics in the workshop.

Results: In the area of *E. coli* and *Salmonella*, the following topics were selected as the most important: Factors influencing shedding, basic understanding of ecology of foodborne pathogens in feed yards, sources/reservoirs of foodborne pathogens, current research and overview of *E. coli* and *Salmonella*, ecology of the organism in the animal and environment, potential of pathogens being harbored in cattle, and biology of the organism in the animal and the environment.

Significance: Data from this research will allow for the development of an effective workshop for feedyard managers and will help decrease the prevalence of foodborne pathogens in the food supply.

P1-104 Developing and Implementing a College-level Course in Home Food Preservation

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Introduction: Many methods of home food preservation can be done by individuals with varied backgrounds and levels of knowledge. In today's society, many undergraduate students more than likely come from environments where they have not been exposed to home methods for processing food or the sciences related to doing it safely. However, at some point they may either be employed in areas where this knowledge and experience would be beneficial or they may become interested in food preservation because of hobbies or lifestyle choices.

Purpose: The objective was to develop, implement and evaluate an undergraduate course on science-based home food preservation methods. The model used was a 3-week, 3-hour-per-day short summer session with daily class meetings and no prerequisite courses required.

Methods: A new three-credit course was offered in the Dept. of Foods and Nutrition. Modules included food chemistry, food microbiology, and preservation techniques including canning, freezing, drying, pickling, fermentation, and jam and jelly production. Fifteen undergraduate students successfully completed the course over two offerings. Lectures and laboratory experiences were supplemented by quizzes and a final exam, an in-depth laboratory report and an independent project that facilitated inquiry training in concepts taught.

Results: Students did improve their knowledge of basic food preservation principles and practices. The average score on the pre-test of knowledge was 51.0%; the average score on the final exam was 88.8%. The average self-rating of their interest in this subject matter increased from 2.6 to 4.3 on a scale of 1 to 5; self-assessment of knowledge gained increased from 1.7 to 4.1 on the same type of 1 to 5 scale.

Significance: This hands-on course with applied home food safety content is an opportunity to teach food science and safety principles as well as knowledge of the food supply not obtained in other courses for most majors. Two-thirds of the students indicated a "very high" likelihood that their food handling and safety practices would change as a result of knowledge learned in this course.

P1-105 Development and Validation of an Instrument to Assess Food Safety Knowledge and Behavior among Low Income Pregnant Women

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Introduction: Pregnant women are at increased risk for certain types of foodborne illness, including listeriosis, which may result in possible loss of the fetus. In the US, a 12 times higher incidence of listeriosis has been reported in Latino pregnant women and their infants vs. their non-Latino conterparts.

Purpose: As part of a multi-state project to document whether food safety education for low-income pregnant women promotes food safety behavior changes and decreased exposure to foodborne pathogens, we developed and validated a self-report instrument designed to assess food safety knowledge, risky food preferences and safe food handling practices addressed in an 8-lesson educational program, Healthy Baby, Healthy Me (HBHM).

Methods: Two sets of educational curricula were developed. The control group HBHM curriculum consisted of the Expanded Food and Nutrition Education Program's 8-lesson curriculum, Eating Smart, Being Active, adapted for pregnant women. The intervention HBHM curriculum had 2.5 additional h of food safety instruction (as 30-minute additions to lessons 3 to 7). All educational materials were made available in English and Spanish. A 48-item pre/post survey was developed based on food safety concepts taught in the curriculum. Questions previously tested for validity and reliability with low-income English-speaking women were used where possible. The survey was evaluated against the curriculum for content validity, then tested for reliability with 29 low income English- and Spanish-speaking women using the test/retest method with no intervention in between. Items with low reliability were eliminated or changed. The curricula and food safety evaluation instruments were then pilot tested in Colorado and Ohio with 18 low income pregnant women. Internal consistency of the questionnaire and of items addressing major constructs was evaluated using Cronbach's alpha.

Results: After careful consideration of test-retest reliability, internal consistency and content analysis results, the final questionnaire consisted of 32 questions, 12 knowledge (alpha = 0.63) and 20 behavior (alpha = 0.79), with an overall Cronbach's alpha reliability of 0.83. The internal consistency of major constructs ranged from 0.22 to 0.83.

Significance: Assuring the validity and reliability of questionnaires is a critical step in the development of any educational project that hopes to produce meaningful results.

P1-106 Assessing the Potential for Cross-contamination in Home Kitchens when Preparing a Meatloaf

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Introduction: Studies of consumer behavior consistently demonstrate undesirable practices during meal preparation, which may lead to the spread of bacteria in the kitchen.

Purpose: This project was designed to assess the extent to which practices used during the preparation of a meatloaf might contribute to microbial cross-contamination.

Methods: Ingredients for the meat loaf were provided to 100 consumers for preparation in their home. Before delivery, the ground beef was mixed with a UV fluorescent compound (FC) that would allow for tracking potential cross-contamination. Upon arrival in the home, participants were asked to put the ingredients away as they would upon returning from the supermarket. They completed a 20-min interview before going to the kitchen and preparing meatloaf as they usually would. Using a checklist, subjects were observed during preparation. After preparation, UV assessments showed the FC in highest amounts in the sink, faucet handles, the countertop, and knife handles.

Results: High levels of FC were noted around fingernails and on backs and palms of hands. Of the 87 persons who washed their hands before handling the meat, only 66 washed for 20 s or more. Only 70 used soap. Four persons dried their hands on their clothes. Two-thirds mixed the meat loaf with their hands; 11 did not wash their hands first. Few persons washed their hands during preparation.

Significance: Many consumers are not practicing the basic steps to prevent cross-contamination. Thus, the potential for spread of bacteria in the kitchen is high. By identifying which food safety practices consumers are failing to conduct adequately, and to what extent bacteria are potentially spread during preparation, targeted educational messages can be developed.

P1-107 Consumers and Food Recalls: What Does the Public Want to Hear?

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Introduction: Motivating people to appropriately respond to a food recall can be extremely difficult. Some consumers are unaware of particular recalls because the right information never reaches them. Others hear about the recall but choose to disregard it, wrongly assuming that the information does not apply to them. Some consumers who are aware of the recall choose to disregard it, and eat the recalled product anyway. At the other extreme, some consumers may avoid the recalled product for months (or years) after the recall has ended. They may also generalize the recall warning to other similar products, or products produced by the same manufacturer, and may change their purchasing and food preparation practices in ways that are not necessarily warranted by the recall.

Purpose: The purpose of this study was to identify public perceptions of food safety and food recalls, and to determine what messages will encourage the public to perform desired responses to recalls such as checking their homes for the recalled product.

Methods: A survey research firm was hired to conduct a national, random-digit dial telephone survey of 1,101 Americans in all 50 states. Data were collected during August and September, 2008.

Results: The findings indicate that 80% of Americans believe that food recalls are becoming more frequent, though far fewer believe that the number of recalls increased between 2006 and 2007. Knowledge about the mechanisms of the food recall system is quite low (e.g., 73% believe that the Food and Drug Administration is responsible for recalls of meat and poultry). More than half of Americans (59%) report having looked in their home for recalled foods at least once, and 35% of these report having found some. When asked to rate the types of information that are most important for the media to include in stories about food recalls, the illnesses and symptoms caused by eating the recalled product and whether anyone has become ill from eating the product were rated as the most important. Similarly, respondents said that knowing how many people had been made ill by a food recall would be most likely to motivate them to check their homes for a recalled food product.

Significance: Taken together, the findings indicate that before paying attention to any of the more detailed information about food recalls, Americans want to determine whether a food recall applies to them (and the food they eat) and the severity of the problem.

P1-108 Education Influences Food Safety Knowledge and Behavior of Pregnant, Low-income English- and Spanish-speaking Women

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Introduction: Vulnerable populations, such as pregnant women, suffer from foodborne illnesses at a disproportionately high rate relative to the general population. Latina women are of particular concern because of their consumption of foods associated with listeriosis.

Purpose: An intervention study assessed the impact of food safety education on the risk of foodborne illness among English- and Spanish-speaking low-income pregnant women. Microbiological exposure was a primary concern.

Methods: Low-income English- and Spanish-speaking pregnant women (age ≥ 18 years) in Ohio and Colorado were recruited to participate in the study and were randomly assigned to two treatment groups: 1) Active Control, 8-session nutrition education program; 2) Experimental, 8-session nutrition education program supplemented with five lessons on food safety. Demographic data was collected pre intervention. Educational outcomes and microbiological samples (food, refrigerator, sink) were measured pre/post intervention. Petrifilms™ were used to quantify aerobic, coliform and *Escherichia coli* counts in sinks and refrigerators.

Results: Preliminary results for 149 subjects have been completed. Regression models for general food safety knowledge ($P < 0.001$, R2 = 0.27) and knowledge of pathogens ($P < 0.001$, R2 = 0.32) show positive pre/post knowledge change in the experimental vs. control group. Younger age, living with a partner, and college education were significantly ($P < 0.05$) related to both measures. Spanish-speakers had reduced consumption of risky foods ($P < 0.001$, R2 = 0.31) and increased handwashing after education ($P < 0.001$, R2=0.24), regardless of treatment group. Coliform counts in sinks were lower in the experimental than control group post education, but in contrast to education outcomes, Spanish subject households receiving education did not experience a reduction in counts ($P < 0.001$, R2 = 0.74). Counts were higher ($P < 0.001$)in Ohio home, than in Colorado, largely accounting for the high R2. Consistent with behavior reflecting underlying preferences, women who had quit smoking during pregnancy and who revealed a low rate of time preference (i.e., willing to delay gratification) lived in an environment with lower microbial counts ($P < 0.05$).

Significance: Preliminary results of this on-going multi-state and interdisciplinary study are encouraging. Significant changes were detected in food safety knowledge and behaviors. Microbiological findings are less robust, possibly due to the high variability in microbial counts, a problem that may be remedied as more observations are recorded.

P1-109 Agrosecurity Awareness Curriculum Design and Training of First Responders to Agricultural and Food Emergencies

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Introduction: Disruption of agriculture and the food supply, by intentional acts or accidental introduction of diseases or contamination, would have devastating consequences. Heightened awareness and planning can improve response, minimizing emergency impacts and shortening recovery time.

Purpose: This project developed, implemented and evaluated a training program to increase agrosecurity awareness and improve recognition of hazards, thus improving response to agriculture and food emergencies.

Methods: The Georgia Agroterrorism Committee identified a need for awareness training. The Agrosecurity Awareness Training: Protecting Agriculture and Food textbook was developed (Brown, C., Choueke, E. and Myers, L.) and used as the basis for outreach education. Eight modules were developed by content specialists and translated by outreach specialists into a format for agricultural responders. Resources for instructors and supplemental activities were developed. Extension Agents were trained in content and implementation. Workshops were conducted statewide by Extension agents and project personnel. Program impact on awareness of agrosecurity issues and responsibilities was evaluated using 11 items and a 5-point Likert scale prior to and following the training.

Results: Participants (1670) included firefighters, law enforcement, personnel from emergency management, wildlife and veterinary, and food industry sectors. For all 11 items, percentages improving awareness ranged from 77% ($P < 0.01$) to 94% ($P < 0.01$). As a result, 85% of participants planned to become familiar with local agriculture and food security

emergency management and response systems. 87% planned to review and remember responses to foreign animal and plant disease outbreaks. 90% planned to become familiar with the Incident Command System. 89% planned to think about preparation in their local communities for handling emergencies.

Significance: This curriculum project can serve as a model for other states and/or countries and has been used as the basis for on-line training modules. Diversity of the audience indicates a need for additional targeted training.

P1-110 Educating Health Care Professionals about *Vibrio vulnificus* Infection

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Introduction: Vibrio vulnificus is a naturally occurring marine bacterium found in high levels during warm weather (April - November), especially along the Gulf Coast. Oysters can accumulate *V. vulnificus* bacteria in their tissues at 100 times the level found in coastal waters. Consumption of raw or undercooked oysters, clams, or mussels may result in *V. vulnificus* infection. Although an average of only 43 cases of these consumption-related infections is reported each year in the United States, the mortality rate exceeds 50%. Raw oyster consumers who are immunocompromised or have other chronic health conditions are at high risk for serious infection.

Purpose: Because health care professionals are highly regarded sources of information, this group was targeted for *V. vulnificus* education efforts and activities beginning in May 2004. Our objective was to develop and implement ways to convey free *V. vulnificus* infection information and resources to health care professionals and encourage them to educate their high-risk patients about their potential for and severity of infection.

Methods: National and regional organizations of health care professionals who treat or advise consumers at high risk for serious *V. vulnificus* infection were identified. Outreach efforts centered on reaching large numbers of medical professionals (physicians, nurses, physician assistants, dieticians) and included development of SafeOysters.org Web site, Internet-based continuing education courses, a health educator fact sheet and multi-media CD, and static *V. vulnificus* displays. These tools were used to provide educational exhibits at meetings/conferences of relevant medical professionals.

Results: From August 2006 to December 2008, educational exhibits were provided at 14 medical meetings with a combined attendance of more than 89,000; in addition, 960 professionals have completed online courses. Since April 2006, the SafeOysters.org Web site has logged more than 200,000 visits. In 2008, the Web site averaged approximately 5,800 visits per month.

Significance: Consumers who are at high risk for serious *V. vulnificus* infection are widely diverse in their health conditions and therefore difficult to reach directly with food safety information. Educating medical professionals who treat these consumers is a more efficient and cost-effective outreach approach

P2-01 Establishing and Improving Process Variation in Quantitative Microbiology with Statistical Process Control Charting

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Introduction: The use of statistical process control charting (SPC) with quantitative food microbiology is relatively new. Prior to the availability of reference cultures with low levels of stable microorganisms, laboratory control samples were primarily aliquots of overnight enrichments with high levels of the target microorganism(s) present. The levels were not representative of routine laboratory samples, were highly variable and offered minimal understanding of laboratory process variations.

Purpose: Control samples prepared from quantified lyophilized cultures can now be used to gain an understanding of the variation associated with microbiological analyses through SPC charting. The SPC data provides objective evidence that a laboratory is operating under control and within the expected variation for a given analysis. In addition, the SPC data provide a straightforward mechanism for the calculation of method uncertainty associated with a quantitative method.

Methods: Comparison of SPC chart data from a network of laboratories within the US during a three-year period was undertaken. Variations of more than twenty different quanitative analyses were compared. Expected method variations were established for each analysis. Expanded use of SPC charting for routine quantitative microbiological analyses using the same lot of lyophilized reference cultures was also implemented in a global network of laboratories spanning 15 countries. Associated method variations were determined for microbiological analyses used to quantitate aerobic mesophilic bacteria, *Staphylococcus aureus*, Enterobacteriaceae, Coliforms, and *E. coli*.

Results: Method variation was reduced overall by thirty percent as demonstrated by the difference between the Upper and Lower Control Limits (Log CFU or MPN) of the SPC charts of more than twenty quantitative analyses in North America. While the variation associated with a given quantitative method was largely dependent on the microbiological technique employed, certain methods were found to be more susceptible to laboratory process differences. For example, results of methods used for the enumeration of *Staphylococcus aureus* were consistent from year to year, while methods used for the enumeration coliforms showed a marked reduction in variation as a result of changes to the testing process.

Significance: This presentation will discuss expected variation for common quantitative food microbiological analyses and will review specific inter-laboratory SPC chart data used in laboratory process improvement efforts.

P2-02 Evaluation of (TA10) Pathogenic Bacterial Multiplex PCR Detection System for Various Food Samples

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Introduction: Salmonella spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are foodborne pathogens capable of causing severe disease. We described the multiplex PCR detection protocol for these pathogens in J. Food Prot. 551-556, (2005).

Purpose: Based on this report, the [TA10] Pathogenic Bacterial Multiplex PCR Detection System was developed as a useful tool for detecting pathogens in food industries.

Methods: The enrichment medium, DNA extraction kit, and multiplex PCR kit were supplied by a Japanese commercial company. The evaluation study was performed in 3 food company laboratories and 2 independent food research institutes. A total of 7 types of food matrices [55 kinds (cheese, apple juice, meat, fresh vegetable, fruits etc…)] of spiked food samples (inoculation level 5 CFU per 25 g) were analyzed with the detection kits, and the results were compared with the results of conventional culture methods in each collaborating laboratory.

Results: The enrichment broth [TA10] was found suitable for enabling all pathogenic bacteria tested to exceed the lowest PCR detection limit (10^3 CFU/ml) within 20 h of incubation at 35°C, and the DNA extraction kit [TA10] in combination with the multiplex PCR detection kit [TA10] provides good recovery and amplification of target DNAs. The detection limit was in agreement (5 CFU per 25 g) within the laboratories, while 7 different types of spiked food samples were tested in three food company laboratories.

Significance: [TA10] Pathogenic Bacterial Multiplex PCR Detection System (included pre-enrichment broth, DNA extraction kit, and multiplex PCR kit) can provide tools for a simple and highly sensitive detection method for pathogens in various food samples. No significant differences in detection sensitivity were seen among the evaluation studies performed in 3 food company laboratories. Therefore, this commercial kit could be a useful tool to help ensure of safety of food for consumers.

P2-03 A Versatile Internal Control for DNA and RNA Real-time PCR Assays

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Introduction: Real-time polymerase chain reaction (qPCR) is a common technique for rapid detection and identification of pathogens in food and clinical samples. However, because of its sensitivity to inhibitors present in the samples analyzed, there is potential for false-negative results, with consequent assay detection failure.

Purpose: The aim of this study was to develop a TaqMan-based internal control system (IC) that could be used as DNA in qPCR or as RNA in reverse transcription qPCR (qRT-PCR) to indicate the presence of assay inhibition and to multiplex the IC with existing bacterial detection methods.

Methods: The internal control (myIC) was designed to be genetically dissimilar to all known organisms and created in a plasmid format with an upstream T7 promoter to generate RNA template as well as DNA. Forward and reverse primers were designed along with a Cy5-labeled TaqMan probe to use in conjunction with the unique template to create a complete internal control set. The myIC system was first tested independently in both DNA and RNA assays and then added to existing qPCR and qRT-PCR bacterial detection methods as an internal control. The multiplexed detection methods were tested against both pure cultures and artificially contaminated produce commodities.

Results: The myIC system was shown to be an effective DNA-based internal control as part of a 4-plex qPCR method for the detection of *Shigella* spp. (pure culture and produce samples) and in a duplex qPCR method for the detection of *Listeria monocytogenes*. It was also a successful RNA-based internal control in the duplex qRT-PCR method of *Salmonella* spp. detection from jalapeno pepper samples.

Significance: Because of the versatility of this internal control, the myIC system can supplement many new or existing qPCR and qRT-PCR pathogen detection methods, greatly reducing the number of false negatives and allowing fast determination of inhibitor presence in various matrices to be tested. While we have utilized myIC only in bacterial assays, its RNA capability makes it a potentially valuable internal control in viral detection as well.

P2-04 Comparison of 3M™ Petrifilm™ Aerobic Count Plate Results for Raw and Processed Meat Samples after 24 Versus 48-h Incubation

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Introduction: The 3M™ Petrifilm™ Aerobic Count (AC) Plate was developed for total aerobic bacteria enumeration in food and is an AOAC INTERNATIONAL Official Method of Analysis (OMA # 990.12).

Purpose: The aim of the study was to compare AC Plate results following an incubation time of 24 h ± 2 h to results obtained by following the official method 48 h ± 3 h incubation time, for raw and processed meat samples (poultry, pig and beef).

Methods: 1024 meat samples were analyzed by five different meat processors, following the same protocol: 10 g of sample + 90 mL of Buffered Peptone Water to prepare the first dilution, followed by 1:10 serial dilutions. 1 mL of each dilution was plated onto AC Plates and incubated first at 35°C ± 1°C for 24 h ± 2 h. Colonies were counted and the results were recorded. These same plates were then incubated for an additional 24 h ± 2 h, and colonies enumerated. Results at 24 h ± 2 h and 48 h ± 3 h were reported as colony-forming units (CFUs)/g of meat.

Results: 1,023 results were statistically analyzed, and results from one representative dilution of each sample were transformed to log base. Regression Analysis was R-Sq = 92.8% and Pearson correlation of log 24 h and log 48 h was 0.963.

Significance: The statistical analysis indicates a strong linear correlation between 24 and 48 h results for AC Plates. The results suggest that naturally contaminated meat samples with aerobic mesophilic flora incubated on AC Plates following a 24 h ± 2 h incubation time may be a valid alternative to the same procedre with 48 h incubation, offering a reduction of 24 h in the incubation time of the AOAC OMA 990.12.

P2-05 Evaluation of Three Methods to Recover Pathogens and Pathogen Surrogates from Whole Muscle Beef Jerky during Drying

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Introduction: The USDA/FSIS Microbiology Laboratory Guidebook for the Examination of Fresh, Refrigerated and Frozen Prepared Meat, Poultry and Pasteurized Egg Products instructs the user to prepare the sample for analysis by stomaching or blending for 2 min.

Purpose: We investigated the recovery of pathogens (*Escherichia coli* O157:H7 and *Salmonella* spp.) and lactic acid bacteria (LAB) pathogen-surrogates (Biosource, *Pediococcus acidilactici*; and Saga 200, *Pediococcus* spp.) from inoculated and marinated whole-muscle beef jerky strips manufactured in a small-scale commercial dehydrator or commercial smokehouse/oven.

Methods: Samples in the dehydrator were dried at 68.3°C (155°F) for up to 8 h; samples in the Alkar oven were dried in a step-wise process: 54.5°C (130°F), 90 min; 60°C (140°F), 60 min; 65.6°C (150°F), 60 min; 70.1°C (160°F), 60 min; 76.7°C (170°F), 60 min. Processes were chosen because they reflect actual industry practice. Samples were taken post-inoculation, post-marination, and at intervals throughout the drying process. Surviving organisms were determined from meat strips homogenized using one of three methods: (1) stomaching for 2 min, (2) pulsifying for 2 min, or (3) dicing, soaking (5 min), then stomaching for 2 min.

Results: Percentage Recovery of pathogens and LABs was calculated relative to post-inoculation or post-marination levels. For the 3 methods, 190, 156, and 190 samples, respectively, were analyzed. Based on post-inoculation levels, survival of LABs was significantly greater than survival of pathogens ($P < 0.01$) for each sampling time and each process, and there was significantly greater recovery of all organisms using homogenization method 3 (dicing/soaking/stomaching) across all conditions (2 processes, 2 marination formulations, 3 bacterial types), ($P < 0.0001$). Recovery with method 3 was enhanced by 0.42 logs for *E. coli* O157:H7, 0.61 logs for *Salmonella* spp., and 0.92 logs for both LABs.

Significance: Care in preparation of meat samples, especially when the product undergoes case-hardening during processing, is important in evaluating the presence of pathogens and pathogen-surrogates in intermediate- or finished-product.

P2-06 Evaluation of a Real-time PCR Assay for Detection of *Listeria monocytogenes* in Combination with New Sample Preparation and Data Analysis Software

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Introduction: Listeria monocytogenes is a human pathogen that is ubiquitous in the environment and can be found in soil, wastewater, animals and vegetation. *L. monocytogenes* is robust and can survive extremes of temperature, pH, and salt concentrations. Detection of *Listeria monocytogenes* is of great importance to the food industry to protect their business and, more importantly, the health of their customers. We created a complete system for the detection of *Listeria monocytogenes* by real-time PCR that includes a lyophilized assay mix, sample preparation kits, instrumentation, and data analysis software.

Purpose: To evaluate the systematic detection of *Listeria monocytogenes* that begins with sample preparation through data analysis.

Methods: Food samples were spiked with and without 1 to 3 CFU *Listeria monocytogenes* and enriched according to standardized methods (25-g sample in 225-mL Buffered *Listeria* Enrichment Broth). Enriched samples were prepared for PCR using 2 sample preparation methods; PrepSEQ™ Rapid Spin Sample Preparation Kit, an PrepSEQ™ Nucleic Acids Detection Kit (both from Applied Biosystems). Real-time PCR was run on the Applied Biosystems 7500Fast instrument using a lyophilized TaqMan® assay mix, and analyzed using Rapid Finder Express software (Applied Biosystems).

Results: The assay showed 100% detection of 50 *Listeria monocytogenes* isolates, and no detection of a panel of 30 non-*Listeria monocytogenes* strains. Early results on 4 food matrices spiked with 1 to 3 CFU showed that the Rapid Finder Express software correctly called 60/60 positive and 24/24 negative samples when samples were prepared with the PrepSEQ™ Rapid Spin Sample Preparation Kit.

Significance: Real-Time PCR technology offers the benefits of rapid results, high sensitivity, and high specificity, as complete systems that include sample prep, software, and instrumentation simplify the workflow.

P2-07 Evaluation of Idaho Technology's R.A.P.I.D.® LT *Listeria* Food Security System in Select Foods and on Environmental Surfaces

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Introduction: The Listeria LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *Listeria* species (*monocytogenes*, *innocua*, *seeligeri*, *welshimeri*, and *ivanovii*) in food and on environmental surfaces. Thermo-cycling takes only 30 min, and the entire procedure takes only 25-29 h. The method involves: a 24-28 hour sample enrichment, bacterial lysis to release DNA, DNA amplification by PCR in the Idaho Technology R.A.P.I.D. LT instrument, internal amplification controls, and automatic result interpretation by the software. Samples can be tested individually or pooled.

Purpose: The Listeria LT FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents for an AOAC evaluation study, in which *Listeria* was spiked into turkey deli meat and Mexican-style cheese, and onto ceramic tile, food-grade stainless steel, and plastic environmental surfaces, and results were compared to results of reference methods.

Methods: Several samples of each food type and environmental surface were tested for *Listeria* with the Listeria LT FSS, MPN analysis, and the reference method tests. The samples were inoculated at levels to result in samples (25 g for food and 4 × 4 inch for surfaces) with approximately 1 CFU of *Listeria* after equilibration. Oxoid ONE broth was used for food and BLEB was used for environmental surfaces. Samples were incubated at 30°C for 24 to 26 h. Samples were tested side by side with the reference method individually, and then pooled and tested.

Results: The Listeria LT FSS is equivalent to the reference methods for turkey deli meat, Mexican-style cheese, ceramic tile, food-grade stainless steel, and plastic in a total of 120 samples. The system detected 54 *Listeria* isolates from all five target species, including 17 different serotypes, and none of 31 non-*Listeria* species were detected. The system is robust and reproducible as demonstrated by ruggedness, lot-to-lot and shelf-life studies.

Significance: This PCR-based system provides reliable detection of *Listeria* in about 25 to 29 h, as opposed to 72 h for the USDA and FDA BAM methods, with fewer steps and minimal sample handling.

P2-08 A Comparative Evaluation of the VIDAS® *Listeria* Species Xpress (LSX) Assay for the Detection of *Listeria monocytogenes* on Environmental Surfaces

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Introduction: Listeria isolated from environmental surfaces in food processing plants can have a detrimental impact on the manufacturing process. Current *Listeria* detection methods for environmental samples are often tedious and labor-intensive. This new screening method, combining the use of a proprietary media, *Listeria* Xpress broth (which reduces lag time and boosts *Listeria* recovery) detects *Listeria* species in food and environmentals within 24 h. The enzyme-linked fluorescent immunoassay (ELFA) utilizes a sealed reagent test strip and Solid Phase Receptacle (SPR) for use on the automated system. Antigens in the sample bind to *Listeria* antibodies coated on the inner wall of the SPR. The system then cycles the sample to detect *Listeria* antibodies that are present in the sample. The wavelength of fluoresced substrate is measured and compared to internal standards. Samples are interpreted as positive or negative.

Purpose: The purpose of this evaluation was to compare the VIDAS® LSX method to the USDA FSIS MLG 8.06 and an immunoassay method utilizing a lateral flow test strip for the detection of *Listeria monocytogenes* on environmental surfaces.

Methods: Twenty-five replicates of each environmental surface, plastic, stainless steel and ceramic were analyzed. The target levels of each strain of *Listeria monocytogenes* used were 0.2 to 2 CFU/100 cm^2. Each 100 cm^2 surface area was inoculated and allowed to dry 16 to 24 h at room temperature (20 to 25°C). The entire sample area of each replicate was sampled with sponges that were enriched in LX Broth and incubated at 30°C for 24 to 26 h. After incubation, a 500 µl portion was added to the sample well of the test strip, heated and analysis initiated. Results were obtained within approximately 73 min. The lateral flow test strip method was performed as per the instructions provided in the package insert and the reference method as per USDA-MLG.

Results: Of 75 samples tested, the new method produced 48 confirmed positive results compared to 43 for the USDA reference method and 48 for the lateral flow test strip method. There was no significant difference between the new method or the lateral flow method and the reference method for all three environmental surfaces, as determined by Chi-square analysis. This new method provided an added advantage for next day screening of *Listeria* species in environmental samples following 24 h proprietary enrichment when compared to the 40 h enrichment for the lateral flow method.

Significance: This new method is an efficient and reliable alternative to the traditional methods of detecting *Listeria* on environmental surfaces.

P2-09 A New "Next Day" Method for Detection of *Listeria monocytogenes* in Food

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Introduction: Detection of *Listeria monocytogenes* in foods with traditional methods is time-consuming, taking up to five days to obtain a negative result.

Purpose: The objective of this study was to determine the performance of a new immunoassay method, VIDAS *Listeria monocytogenes* Xpress (LMX), for the next-day detection of *Listeria monocytogenes* in food samples.

Methods: The detection method is associated with a specifically formulated LMX broth containing optimized concentrations of selective agents to inhibit competitive bacteria. For the food study, samples were culturally enriched for a total of 26 h in LMX broth, before testing in the VIDAS instrument. Positive results were confirmed by streaking enrichment cultures onto selective chromogenic agar. The new method was compared to the ISO 11290-1 reference method.

Results: The detection limit, established with 50 *L. monocytogenes* strains, was found to be between 2×10^3 and 3×10^5 CFU/ml in LMX broth. No cross reaction was observed with 30 potentially interfering strains at the growth level reached in a non selective medium. The food study included 370 food products (238 meat, 87 dairy and 45 seafood products). 153 samples were confirmed positive by at least one of the methods 23 by the immunoassay only, 17 by the cultural method and 113 by both methods). Sensitivity was 88.9 % for the immunoassay and 85.0 % for the reference method. Difference observed between the two methods was not statistically significant. Agreement between the two methods was 89.2%. As all positive results were confirmed after subculture, the test specificity was 100%.

Poster

Significance: This study demonstrated that the VIDAS LMX method is comparable to the ISO 11290-1 method for the recovery of *Listeria monocytogenes* in meat, dairy and seafood products. It provides a very rapid, sensitive and convenient way of obtaining a presumptive result within 27 h of sample setup.

P2-10 Rapid and Simultaneous Detection of *Salmonella* spp. and *Listeria monocytogenes* in a Poultry Processing Plant by Multiplex-PCR

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Introduction: Environmental sampling by bacteriological methods requires many days to obtain results for decision making. Multiplex-PCR is a cost-saving method for the simultaneous detection of foodborne pathogens such as *Salmonella* spp. and *Listeria monocytogenes*. The bacteriological detection method takes 5 days for *Salmonella* spp. detection and 14 days for *Listeria monocytogenes*, while Multiplex-PCR takes 18 h for both pathogens. Enviromental samples were obtained after cleaning and sanitation activities in a poultry processing plant in Jalisco, México.

Purpose: To rapidly and simultaneously detect foodborne pathogens in a poultry processing plant, between the period of cleaning and sanitation and the time of processing activities.

Methods: Twenty cotton swab samples with 0.1% of peptonated water were used to swab a 100 cm^2 surface (10 cm × 10 cm) on sequential points. The detection method consisted of sample incubation at 35°C for 12 h, followed by Multiplex-PCR.

Results: 11/20 (55%) of the samples tested positive for *Salmonella* spp. and 6/20 (30%) tested positive for *Listeria monocytogenes*. Samples that tested positive for *Salmonella* spp. were: chiller, knife, apron, gloves, small box, packing floor, behavee 3, carcass crate, paste production floor, fresh production floor and shipping floor. Samples that tested positive for *Listeria monocytogenes* were: knife, apron, gloves, small box, packing floor and behavee 3.

Significance: The samples that tested positive for *Salmonella* spp. and *Listeria monocytogenes* indicate a low cleaning and disinfection process efficiency. A rapid and simultaneous foodborne analysis, like Multiplex-PCR, could be applied on a weekly basis for routine sanitation evaluation, so that good corrective actions can be taken opportunely.

P2-11 Comparison of Two Polymerase Chain Reaction (PCR) Kits and an Immunoassay against ISO 6579 for the Detection of *Salmonella* in Foods

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Introduction: One of the most important foodborne pathogens tested for by laboratories is *Salmonella*. Traditional culture-based methods take a minimum 3 days to obtain negative results and several days to confirm positive results. In contrast, some commercial tests have greater specificity and offer the potential to significantly reduce test times (to at little as 29 h).

Purpose: This study compared the ability of 3 commercial test kits against a standard culture method (ISO 6579) to detect strains of *Salmonella* in pure culture (specificity) and in foods.

Methods: Three commercial kits, TaqMan *Salmonella enterica* detection kit (Applied Biosystems), BAX Q7 Salmonella detection kit (DuPont Qualicon) and Transia Plate Salmonella Gold ELISA (BioControl Systems Inc.), were used in accordance with the manufacturer's instructions. The specificity of each kit was established with 22 *Salmonella* strains (inclusivity) and 15 non-target organisms (exclusivity) in pure culture. Nine food matrices (36 samples) were contaminated with low levels (range 2 to 10 CFU/25 g), and 9 uninoculated samples were used to compare detection against the standard method (ISO 6579).

Results: The TaqMan PCR, BAX PCR and ELISA tests gave 21, 22 and 20 positive results (inclusivity), respectively. All non-target organisms were negative (exclusivity) by these tests. Tests with spiked samples yielded 33 positive results, which was in agreement with the results of the standard method, although only the TaqMan PCR and ELISA test were in full agreement with the *Salmonella* reference method ISO 6579.

Significance: This study highlighted the potential benefit of using rapid qualitative methods for the detection of *Salmonella* in foods.

P2-12 Comparison of a Standard Culture Method and a Real-time PCR Assay for Detection of *Salmonella* in Foods with Different Levels of Background Flora (Boiled Pork and Broccoli Sprouts)

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Introduction: Salmonellosis is one of the most infectious diseases in the world and a common cause of gastrointestinal food poisoning. Recent *Salmonella* outbreaks have been associated with fresh vegetables, including fresh sprouts. However, there are few studies on the application and evaluation of rapid detection methods such as real-time PCR for detection of foodborne pathogens in foods with high levels of background microflora, such as sprouts.

Purpose: The objectives of this study were to compare the sensitivity of culture methods and real-time PCR to detect *Salmonella* in food samples with different level of natural background flora; boiled pork (low level) and broccoli sprouts (high level).

Methods: Real-time PCR assay was evaluated for the detection of major foodborne pathogens, *Salmonella* Enteritidis (SE) and Typhimurium (ST), in broccoli sprouts and boiled pork and compared to detection with the standard culture method. The bulk samples (500 g) of each food were inoculated with various concentrations of inoculums prepared, and then divided into 20 samples (25 g each) to generate partial positive samples in a separate experiment for each of the pathogens. Negative controls, uninoculated food were also prepared. The level of background flora of each food samples were calculated at each trial. All of the inoculated and uninoculated control samples were analyzed by both real-time PCR and culture method.

Results: Aerobic plates showed that counts for the level of background flora were lower in boiled pork than in broccoli sprouts, less than 100 CFU/g and 6.7×10^7 (± 1.5) CFU/g, respectively. In boiled pork, the culture method and real-time PCR detected *Salmonella* equally well, and a statistically significant difference was not observed between the two methods. In broccoli sprouts, the culture method identified 16 out of 80 samples (20%) as positives, and real-time PCR similarly identified 39 out of 80 samples (49%) as positives. With regard to this result, obtained from 4 trials, an extremely statistically significant difference was observed between the two methods.

Significance: The real-time PCR assay was less affected by background flora and more sensitive in detecting low numbers of *Salmonella* than the culture method, particularly in the sample with a high degree of background flora. For further improvement of the culture method and real-time PCR, additional measures will be needed to increase the number of *Salmonella* to detectable levels in samples with high levels of background flora to minimize the effects of background flora.

P2-13 Washing and Enrichment of Jalapeño Peppers Using Small Volumes of Non-selective Broth Facilitates Rapid Cytometric Detection of *Salmonella* Saintpaul

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Introduction: The 2008 outbreak of salmonellosis associated with the consumption of jalapeño and Serrano peppers imported from Mexico highlights the need for rapid methods for detection of this pathogen on this and other types of fresh produce. Although enrichment of produce represents a bottleneck, it may be possible to reduce overall assay time through the use of improved methods for sampling and sample preparation prior to enrichment.

Purpose: The purpose of this study was to develop a rapid, streamlined method for detection of *Salmonella* ser. Saintpaul on jalapeño peppers. Elements evaluated included use of a larger analytical unit and a one-step approach for wash-based removal of bacteria with delivery into a concentrated enrichment volume.

Methods: Jalapeño peppers were spot inoculated with stationary phase cells of *S.* Saintpaul ATCC 9712. After drying (~3 h), cells were detached via pulsification into 10 ml Trypticase Soy Broth (TSB) for non-selective, low-volume enrichment at 37°C. One-milliliter aliquots were taken at regular intervals during enrichment and examined via combined fluorescence *in situ* hybridization (FISH) and flow cytometry. Use of a larger analytical unit (4 peppers, ~100 g), and use of low-volume washing with 0.1% peptone water (PW) for direct detection of *S.* Saintpaul were also examined.

Results: Pulsification enabled rapid, vigorous and non-destructive washing of jalapeño surfaces, facilitating removal and concentration of attached bacteria into small volumes of TSB or PW. Using FISH and flow cytometry, we were able to detect 10^3 CFU g^{-1} *S.* Saintpaul directly in PW, or as few as 10 CFU g^{-1} after only 6 h of enrichment in TSB. Instead of being limited to the testing of individual peppers, we found that we could also analyze a larger analytical unit (4 peppers, ~100 g) using this method.

Significance: We have developed a simple "FISH and flow" method for rapid and sensitive detection of *S.* Saintpaul from jalapeño peppers. The method takes advantage of the thorough but non-destructive nature of Pulsifier-based washing for removal of bacteria from pepper surfaces, combined with dual use of small volumes of non-selective media as both a wash and as a growth medium. Because total assay time (wash-based removal of cells, cell fixation, hybridization and flow cytometry) was ~2 h, our method may enable the detection of low numbers of salmonellae on jalapeño surfaces within a single eight hour work shift. This approach provides the fresh produce industry with a rapid tool for monitoring jalapeño peppers for contamination with *Salmonella*, and may also be useful for rapid detection of this pathogen in other types of fresh produce.

P2-14 Evaluation of PCR Detection of *Salmonella* in Alfalfa Sprouts and Spent Irrigation Water Collected during Sprouting of Naturally Contaminated Seeds

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Introduction: Traditional *Salmonella* confirmation methods can take up to four days, and high levels of background flora, if present, often interfere with the isolatation of typical colonies from selective agar plates. PCR-based detection is a more sensitive method that produces results in shorter time. Our previous work revealed that sprout irrigation water spiked with low numbers (1 to 100 CFU) of *Salmonella* enriched overnight in tetrathionate (TT) broth and buffered peptone water with novobiocin (BPW+n) was able to detect *Salmonella* using a PCR detection system, but few studies using PCR for detection of *Salmonella* in naturally contaminated products have been published.

Purpose: This study evaluated the efficacy of a PCR-based system (DuPont Qualicon BAX) using two enrichment protocols for detection of *Salmonella* in sprouts and spent irrigation water collected during sprouting of seeds naturally contaminated with *Salmonella*.

Methods: Alfalfa seeds naturally contaminated with *Salmonella* were grown in jars at 20°C and 30°C for three days. Afterwards, five samples of spent irrigation water and five samples of sprouts were enriched overnight individually in TT and BPW+n at 42°C and in lactose broth (FDA BAM) at 37°C. Levels of *Salmonella* present in the spent irrigation water and sprouts were determined by MPN analysis, and both were plated on PCA to determine background flora levels. After enrichment in TT and BPW+n, the samples were run in the BAX system and results compared to the FDA BAM method. Three trials for each growth temperature were done.

Results: Salmonella levels were lower at 20°C than at 30°C for some trials, and background flora levels ranged from 10^7 to 10^8 CFU/g or ml at 20°C and 10^8 to 10^9 CFU/g or ml at 30°C. In trials with a *Salmonella* level >1.1 MPN/g or ml, both the BAX and FDA BAM methods were able to detect positives in all samples. In trials with lower levels of *Salmonella*, BAX was able to detect more positive samples than FDA BAM. For one trial with < 0.003 MPN/g or ml of *Salmonella*, the presence of the pathogen was not indicated by either the BAX or the FDA BAM method.

Poster

Significance: The results suggest that a PCR system such as BAX is a faster and more sensitive method for detecting low levels of *Salmonella* in sprouts or spent irrigation water collected from sprouting of naturally contaminated seeds.

P2-15 Comparison of Commercial RNA Extraction Kits for Preparation of DNA-free Total RNA from *Salmonella* Cells

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Introduction: The isolation of DNA-free RNAs is a critical step for conducting RT-PCR assays. Nearly every RNA extraction procedure described previously has resulted in RNA samples being contaminated with genomic DNA. The consequence of this is, of course, a false positive result after RT-PCR. This is true, in particular, when real-time RT-PCR assays are employed for high sensitivity applications where false-positive results equate generally with a failure of the assay

Purpose: The aim of this study was to compare some of the most commonly employed RNA extraction kits and a classic plasmid extraction procedure for determining which provides:(1) superior RNA recovery (e.g., the amount of RNA), (2) optimal purity (e.g., DNA contamination), (3) ease of deployment for high-throughput analysis and (4) the lowest price per reaction.

Methods: RNA was extracted from exponential Salmonella SE5 cells using: RiboPure™-Bacteria Kit (Ambion), PureLink™ RNA Mini Kit (Invitrogen), UltraClean™ Microbial RNA Isolation Kit (MoBio), RNeasy Mini Kit (Qiagen), MasterPure™ RNA Purification Kit (EPICENTRE), and Plasmid DNA Isolation Reagent System (Carolina Biological). RNA concentration and initial purity was assessed through A260/280 ratio using Nanodrop. Further analysis for detecting genomic DNA contamination was performed by a quantitative reverse transcriptase real time PCR (RT-qPCR) targeting invA mRNA.

Results: The purity of the RNA obtained with all of the kits, as assessed by the A260/280 ratio, was highly comparable at around 2.0. A higher total RNA concentration was obtained with the kit from EPICENTRE and with the plasmid extraction kit; however, DNA contamination (A280) was markedly high with these methods. Following in RNA recovery yield were the Invitrogen and Qiagen kits. RT-qPCR analysis of the samples before and after DNase I treatment showed that most kits performed similarly for invA mRNA detection save for the EPICENTRE and Mo Bio kits where DNA contamination was higher and interfered with the accuracy of the assay.

Significance: This comparison showed that most of the kits analyzed are suitable for *Salmonella* RNA extraction. A higher RNA recovery was achieved with the EPICENTRE kit, but this kit also co-isolated high levels of genomic DNA which interfere with invA mRNA detection by RT-qPCR. Based on total RNA yield, levels of contaminant DNA, feasibility of high-throughput analysis, and price per reaction, the Qiagen and Invitrogen kits were shown to be the optimal choices for detection of invA mRNA by RT-qPCR. The use of these kits should greatly reduce the amount of false positives (i.e., DNA contamination) and will allow for a fast determination of invA mRNA in food samples.

P2-16 Assessment of Rapid Pathogen Detection Kits for *Salmonella* on Melons for Test to Release Programs

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Introduction: Occasional discrepancies between contract labs and regulatory labs in detection and recovery of *Salmonella* from melons, from a given lot, may be a reflection of non-homogeneous contamination, differences in general methods, and more recently, specific inherent differences between conventional and rapid test kit sensitivities.

Purpose: To evaluate the specificity and sensitivity of rapid test kits considered for detection of *Salmonella* within 'Test and Release' protocols on cantaloupes and honeydews.

Methods: Five platforms of combined recovery and detection were used: PATHATRIX, BAX®, Assurance GDS™, RapidChek® and Reveal®. Melons were surface inoculated with 36 or 100 CFU/melon of a three serovar cocktail of *Salmonella enterica*. After 5 days storage at 5°C, melons were peeled and 25 g, including the inoculated rind-spot, was subjected to the recommended protocol. In addition, over 200 non-inoculated melons were surveyed for *Salmonella* using the first three detection methods listed.

Results: With 36 CFU/fruit, on cantaloupe, there were 100, 66 and 33 percent detection for BAX, PATHATRIX and RapidChek respectively. Reveal and GDS were negative for all inoculated melons. For honeydew, all test results were negative. At 100 CFU/fruit, 88% of cantaloupes were positive using BAX whereas 28% were positive, from the same enrichment culture, using GDS. All uninoculated controls were negative. Additional viability-confirmation controls demonstrated that honeydew had comparatively poor survival of *Salmonella*. In the expanded survey with uninoculated melons, 5 confirmed false positives were observed with BAX® on cantaloupes.

Significance: Our outcome of approximately 2% 'false positives' would likely create management challenges if applied to Test to Release programs for cantaloupe, without protocol modification. With further melon-specific performance data, the sensitivity threshold for a positive detection outcome, in some regions, may be safely elevated to eliminate most false positive reactions. False negatives, especially with low levels of stressed cells, also remain an issue to resolve for routine compliance testing using rapid methods.

P2-17 An Independent Evaluation of a New Method for the Detection of *Salmonella* in a Variety of Foods: The VIDAS ® Easy *Salmonella* Assay

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Introduction: Salmonella is one of the main causes of food poisoning; thus the rapid detection of this pathogen is a vital component of food safety. This new screening method detects *Salmonella* spp. in food and environmentals samples within 48 h. The alternative method utilizes a simple 2-step enrichment procedure for use with the enzyme linked immuno-fluorescent assay (ELFA). In the new enrichment protocol, a single selective proprietary broth (SX2) replaces the two broths

in traditional methods and eliminates the need for transfer to M broth, incubation and subsequent pooling of M broths prior to ELfA screening.

Purpose: The purpose of this AOAC-RI™ independent evaluation was to compare the new method to the FDA BAM in liquid eggs, ice cream and orange juice and to the USDA FSIS MLG 4.03 method in turkey for the detection of *Salmonella* species.

Methods: Twenty-five replicates each of liquid eggs, ice cream, orange juice and turkey were analyzed. The target level of each strain of *Salmonella* used for challenging the three matrices was 1 to 10 CFU/25 g. Following incubation at 35°C for 16 to 22 h, a 0.1 ml aliquot of primary enrichment was transferred to 10 ml of SX2 broth and incubated at 41.5°C for 22 to 26 h. After incubation, samples were heated by both boiling and Heat and Go processes and a 500 µl portion was added to the sample well of the test strip. The assay was initiated and results obtained within 45 min. Replicates were streaked onto chromogenic SM2 agar and reference method selective media. Final confirmations were achieved by VITEK® AOAC OMA Method 991.13. The reference methods were performed as per the FDA BAM and USDA-MLG.

Results: Of 100 samples tested, the new method produced 35 confirmed positive results, compared to 34 for the reference methods. There was no significant difference between the new method and the reference methods for all four foods, as determined by Chi-square analysis at the 95% probability level.

Significance: This new method demonstrates reliability as an alternative screening method for the rapid detection of *Salmonella* in a variety of foods.

P2-18 A Comparative Evaluation of the VIDAS ® Easy *Salmonella* Assay for the Detection of *Salmonella* in Food and Poultry Rinse

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Introduction: Current *Salmonella* methods are often time consuming and can take up to 5 days to obtain a negative result. This new screening method detects *Salmonella* spp. in food and environmentals in less than 40 h. The alternative method utilizes a simple 2-step enrichment procedure for use with the enzyme linked immuno-fluorescent assay (ELFA). In the new enrichment protocol, a single selective proprietary broth (SX2) replaces the two broths in traditional methods and eliminates the need for transfer to M broth, incubation and subsequent pooling of M broths prior to ELfA screening.

Purpose: The purpose of this evaluation was to compare the VIDAS® Easy *Salmonella* method to the USDA FSIS MLG 4.04 and an immunoassay method utilizing a lateral flow test strip for the detection of *Salmonella* spp. in poultry.

Methods: Twenty-five replicates each of cooked turkey, raw ground turkey and poultry rinse were analyzed. The target levels of each strain of *Salmonella* used for challenging the three matrices were 0.2 to 2 CFU/g. Replicates of cooked turkey and raw ground turkey were enriched in BPW and poultry rinse in 2XBPW and incubated at 35°C for 16 to 22 h. A 0.1 ml aliquot of primary enrichment was transferred to 10 ml of SX2 broth and incubated at 41.5°C for 22 to 26 h. A 500 µl portion was added to the sample well of the test strip and heated by Heat and Go at 131°C for 15 min, cooled and loaded into the system. The assay was initiated and results obtained within 45 min. Replicates were streaked onto chromogenic SM2 agar and reference method selective media. The lateral flow test strip method was performed as per the instructions provided in the package insert and the reference method as per USDA-MLG. Final confirmations were performed by VITEK® AOAC OMA Method 991.13.

Results: Of 75 samples tested, the new method produced 40 confirmed positive results, compared with 41 for the USDA reference method and 32 for the lateral flow test strip method. There was no significant difference between the new method and the reference method for all matrices tested, including cooked turkey, raw ground turkey and poultry rinse, as determined by Chi-square analysis. There was a significant difference between the lateral flow method and the reference method for poultry rinse.

Significance: This new method is an accurate, reliable detection assay and can be a favorable alternative to traditional reference methods of detecting *Salmonella* species in food, with presumptive results available on the second day after sample set up.

P2-19 Compositing Produce Rinse Samples for Increased Throughput for Real-time PCR Detection and Recovery of *Salmonella* and *Escherichia coli* O157:H7 in Artificially Contaminated Produce

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Introduction: Salmonella spp. and *Escherichia coli* O157:H7 have been responsible for a number of foodborne disease outbreaks associated with fresh produce. Detection and isolation of these pathogens is time consuming and labor intensive. High throughput for sample analysis of multiple pathogens could be improved with the use of a universal rinse and compositing protocol.

Purpose: To assess the effect of compositing rinsates prepared from leafy vegetable samples on the sensitivity of detection and recovery of *Salmonella* Typhimurium and *Escherichia coli* O157:H7. Spinach, Romaine lettuce, green onions, basil and cilantro were used as food matrices.

Methods: For each type of produce, one of five samples (500 g) was inoculated with *Salmonella* Typhimurium and *E. coli* O157:H7. Three inoculation levels were evaluated: approximately 0.1 CFU/g, 1 CFU/g and 10 CFU/g produce. Samples were rinsed with Butterfield's Phosphate Buffer and rinsate portions were tested. For compositing, each contaminated rinsate was combined with four uncontaminated rinsates. Individual and composited sample rinsates

were enriched for *Salmonella* and for *E. coli* O157 in double strength enrichment broth. Samples were screened for the pathogens by real time PCR. Recovery of inoculated pathogens was performed by streaking enrichments on TC-SMAC and Rainbow agar for EHEC and on Hektoen agar for *Salmonella*. Isolates were confirmed by real-time PCR.

Results: Salmonella and EHEC were detected and isolated from individual and composited samples prepared from inoculated spinach, green onions, basil, and Romaine lettuce rinsates prepared from the inoculated produce items at all inoculation levels. For cilantro samples, good recovery and isolation was seen for both EHEC and *Salmonella* in the individual samples at all inoculation levels but sensitivity was reduced to approximately 1 CFU/g produce with the composited samples.

Significance: Compositing sample rinsates worked well with most produce items. Items with high background levels, had reduced sensitivity with a composited analysis.

P2-20 Testing for *Salmonella* and *E. coli* O157:H7 from a Single 8-h Enrichment

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Introduction: Most *Salmonella* and *E. coli* O157:H7 outbreaks are linked to two food types: fresh produce and beef. Traditional testing protocols call for separate enrichment methods when testing for *Salmonella* versus testing for *E. coli* O157:H7 in these matrices.

Purpose: The objectives of this study were two-fold; one was to investigate using an established 8-h beef enrichment method with the BAX® system PCR method for detecting *E. coli* O157:H7 in fresh produce and the other was to evaluate the same enrichment with the same PCR method for detecting *Salmonella* in both beef and produce.

Methods: Produce was spiked with *E. coli* O157:H7, and beef and produce were spiked with *Salmonella* at target levels set to yield fractional positive results. Samples were evaluated using the appropriate culture-based reference method and the PCR test kit method following the 8-h enrichment protocol. Twenty spiked and five unspiked samples per food type per method were tested and compared.

Results: Statistical analysis on both *E. coli* O157:H7 and *Salmonella* in all matrices indicated the test method performed as well as or better than the reference method for detecting both *E. coli* O157:H7 and *Salmonella*.

Significance: This approach demonstrates that both *Salmonella* and *E. coli* O157:H7 can be detected from the same 8-hour enrichment, which may save food companies cost, time and labor through reductions in sample preparation, media preparation, incubator space and waste streams.

P2-21 Sensitivity and Specificity Testing of the New *Escherichia coli* O157:H7 PCR-based Detection Assay Using Idaho Technology's R.A.P.I.D.® LT Food Security System

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Introduction: Escherichia coli serotype O157:H7 continues to be a serious health risk and a costly concern for the food industry. Idaho Technology Inc. has developed a rapid PCR-based multiplex assay for the detection of *E. coli* O157:H7 in food. The *E. coli* O157:H7 assay will be the newest addition to the R.A.P.I.D. LT Food Security System (FSS). This assay uses real-time PCR to detect the O157 serotype and a post-amplification melt analysis to differentiate the O157:H7 from O157:non H7 products. Sequence-specific fluorescent probes are used in both the PCR and melt analysis to add an additional level of specificity to the assay. In addition, the assay contains an internal control that is read in a separate fluorescent channel.

Purpose: The purpose of this study was to evaluate the sensitivity and specificity of the *E. coli* O157:H7 RAPID LT FSS assay.

Methods: Sensitivity and specificity were determined using purified DNA quantified by A260 measurements. Sensitivity was determined using serial DNA dilutions with 2 users and 4 instruments. The sensitivity or limit of detection (LOD) was defined at 85% success and 90% confidence. Inclusivity was performed at 10 pg (~1,600 copies)/reaction with a panel of 340 *E. coli* O157:H7 isolates from various sources including the USDA Clay Center, Marshfield Food Safety Clinic and ATCC. Exclusivity was performed at 1 ng (~160,000 copies) per reaction with 171 non-(O157:H7) *E. coli* isolates of various O- and H- serotypes and 54 non- *E. coli* near neighbor isolates (225 total).

Results: The LOD of the assay was determined to be 16 copies per reaction. All 340 O157:H7 isolates were identified by real-time PCR and generated melting peaks in the expected range of 59.5 to 62.2°C for the O157:H7 serotype. Of the 171 non-(O157:H7) isolates, 21 were O157-non H7 and were identified by real-time PCR but generated melting peaks within a range of 55.6 to 57.3° C. The difference in melting peak ranges defines O157:H7 from O157:non-H7 serotypes. Four isolates previously defined as O157:non motile (2) and O157:H-unknown (2) were identified by real-time PCR and melted in the O157:H7 serotype range, suggesting that these isolates are genetically the same as O157:H7. The remaining 200 isolates were not detected by PCR and did not generate a melting peak.

Significance: These data suggest that this PCR-based assay is sensitive and specific for the detection of *E. coli* O157:H7. Current efforts are focused on validating this assay for AOAC approval with the associated enrichment protocols and using these melting peak ranges to enable the FSS software to assign accurate result calls for the *E. coli* O157:H7 RAPID LT FSS assay.

P2-22 Detection of *Escherichia coli* O157:H7 in Alfalfa Sprouts by Real-time PCR Combined with Immunomagentic Separation with and without an Acid Treatment

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Introduction: E. coli O157:H7 has been implicated in foodborne disease outbreaks associated with alfalfa sprouts. However, isolation of the pathogen from sprouts by standard cultural methods can be difficult because of the high background microflora.

Purpose: This study evaluated an improved procedure for rapid detection and isolation of *E. coli* O157:H7 from sprout samples.

Methods: Alfalfa sprouts were artificially contaminated with *E. coli* O157:H7 at low (~0.2 CFU/g) and high (~2 CFU/g) levels and enriched in modified buffered peptone water + pyruvate (mBPWp) with cefsulodin and vancomycin at 42°C with shaking. After 5 h and 24 h, the target organisms were concentrated by IMS using PATHATRIX™ and Dynabeads® MAX *E. coli* O157. IMS beads were screened by real-time PCR for simultaneous detection of *stx*1, *stx*2 and uidA genes, using the SmartCycler. Additionally, broth cultures and IMS beads were streaked onto selective agar plates (Rainbow®agar, R&F® *E. coli* O157 Chromogenic medium and TC-SMAC agar) for isolation of *E. coli* O157:H7. Both broth cultures and IMS beads were also acid treated to improve upon cultural recovery.

Results: After 5 h enrichment and PATHATRIX™ IMS, *E. coli* O157:H7 was detected by real-time PCR in 23/25 samples inoculated at the low level, and isolated from 7/25 inoculated samples on selective agars, but from 22/25 following acid treatment of the beads. *E. coli* O157 was detected in 22/25 samples and isolated from 0/25 following IMS with the Dynabeads® MAX *E. coli* O157 system, but from 15/25 following acidification of the beads. After 24-h enrichment, cultural recovery was also improved for enrichment broths and both types of IMS beads following acid treatment of the samples.

Significance: Acidification of *E. coli* O157 enrichment broths and IMS beads improved isolation by eliminating competing organisms that make isolation difficult.

P2-23 Development and Characterization of Monoclonal Antibody Specific for *Escherichia coli* O157:H7

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Introduction: Escherichia coli O157:H7 is one of most harmful foodborne pathogens and causes hemolytic uremic syndrome and hemorrhagic colitis in humans. Therefore, rapid methods for *E. coli* O157:H7detection is required to prevent outbreaks of food poisoning.

Purpose: Objectives of this study were to produce new monoclonal antibodies specific to *E. coli* O157:H7, to estimate their specificity, and to develop an indirect ELISA for the rapid detection of *E. coli* O157:H7.

Methods: To immunize mice, heat-killed *E. coli* O157:H7 was prepared and used as an immunogen. The mice showing high titer were used for cell fusion and cloning. The characterization of monoclonal antibodies (MAbs) produced from hybridoma cells obtained were confirmed by enzyme linked immunosorbent assay (ELISA) and Western blot. The specific MAb was used to develop an indirect ELISA for rapid detection of *E. coli* O157:H7.

Results: A MAb was produced from a 4G8-6 hybridoma cell that was developed by cell fusion and cloning and was specific to *E. coli* O157:H7 but showed little cross-reaction to *Listeria monocytogenes* in ELISA and Western blot testing. The detection limit of indirect ELISA base on 4G8-6 MAb was 10^6 CFU/ml, and procedures of assay were completed within 4 h. The indirect ELISA has potential as a rapid and cost-effective screening tool for the detection of *E. coli* O157:H7.

Significance: Since outbreaks of bacterial infection associated with foods and drinks have become more frequent, rapid methods for foodborne pathogens from food are needed. These results indicate that the developed indirect ELISA could be a useful tool for rapid screening of *E. coli* O157:H7 in various foods.

P2-24 RapidChek® SELECT™ *E. coli* O157 Test System for the Detection of *Escherichia coli* O157 in Meat Products

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Introduction: Cattle have been identified as a major reservoir of *Escherichia coli* O157 and foods of bovine origin, including beef and dairy products, have been implicated in many outbreaks of infection worldwide. Since *E. coli* O157 has an extremely low infective dose, with fewer than 50 viable cells capable of causing illness, it is particularly pertinent that it can be detected in foods when present in low levels. Thus, sensitive, fast, and reliable detection methods are needed to monitor foods for *E. coli* O157 and ensure that safe food is being supplied to the consumer.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® SELECT™ *E. coli* O157 test system against the ISO reference method (NF EN ISO 16654:2001) for the detection of *E. coli* O157 in meat products.

Methods: A method comparison study was conducted to determine accuracy, specificity, sensitivity, and relative detection level. Sixty-three samples were analyzed by both methods. Selectivity was evaluated by testing 50 target microorganisms and 30 non-target microorganisms.

Results: The test system demonstrated 89% relative accuracy. The relative sensitivity of the method was 87% and 90% for 8 and 24 h, respectively. The relative specificity was 91% and 88% for 8 and 24 h, respectively. The relative level of detection of the test method varies from 0.74 to 1.57 CFU/25 g while the limit of detection of the reference method varies from 0.77 to 1.64 CFU/25 g. The selectivity of the method was satisfactory.

Significance: The target pathogen can be detected at very low levels of contamination in as few as 8 h with the test system. Confirmation of a positive result can be accomplished in as little as 2 days with the test method versus 3 to 4 days with the reference method.

P2-25 Validation of the Reveal® 8-hour and 20-hour Methods for Detection of *Escherichia coli* O157:H7 in 375-g Beef Samples

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Introduction: Lateral flow or immunochromatographic assays are routinely used by the beef processing industry in testing for *E. coli* O157:H7 contamination. A common approach is to composite up to 15-25 g test samples into one 375 g sample for analysis. This allows more testing to be done while controlling testing costs. While several methods, including the Reveal methods, have been extensively validated and have achieved AOAC method status, to this point validation of methods specifically for 375 g samples has been limited.

Purpose: The purpose of this study was to validate the Reveal 8-hour and 20-hour methods for use with 375 g ground beef and beef trim samples and to compare performance of the Reveal methods with that of the USDA-FSIS reference culture procedure.

Methods: Product was inoculated with low levels of *E. coli* O157:H7 to yield fractional positive data sets, i.e., a target of 5 to 15 positive samples out of 20 replicates. Beef was inoculated in bulk and then divided into 40 portions, 20 each for the Reveal and reference methods. Enrichment for the reference method was conducted according to the procedure in the FSIS Microbiology Laboratory Guidebook, except that a screening method was not used; all enrichments were plated for confirmation. The Reveal methods were performed according to the manufacturer's instructions, using either 8-h or 20-h enrichment media as specified. As it was anticipated that an enrichment period longer than 8 h might be required, testing was performed at several time points, including 8, 10, 11 and 12 h. All samples were plated for confirmation.

Results: The 20-hour Reveal and reference methods produced the same number of confirmed positives for the ground beef samples. For beef trim, the Reveal method produced more positives than the reference procedure, but this difference was not statistically significant. For the 8-h method, results statistically equivalent to the reference method were achieved after 8, 10 and 11 h of enrichment for ground beef and after 10 or 11 h of enrichment for beef trim, but maximum method sensitivity was only achieved after 12 h of enrichment. For both sample types, the Reveal methods produced more positives than the reference culture procedure, but in neither case were these differences statistically significant. Test specificity for both Reveal methods was 100%.

Significance: Results of this study showed that the Reveal 20-h and 8-h methods (with extended enrichment) are highly sensitive and specific when applied to 375 g beef samples. The new procedures have been approved as modifications to AOAC Official Methods 2000.13 and 2000.14.

P2-26 A New Immunoassay Method for the Simultaneous Detection of *Escherichia coli* O26, *Escherichia coli* O111 and *Escherichia coli* O157:H7

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Introduction: Even though enterohemorrhagic *E. coli* (EHEC) have been demonstrated at the origin of numerous foodborne outbreaks, current detection methods target only *E. coli* O157:H7.

Purpose: The goal of this work was the development and preliminary evaluation of a new immunoassay for the simultaneous detection of 3 important EHEC serogroups, *E. coli* O26, O111 and O157:H7.

Methods: The assay developed associates both monoclonal antibodies and recombinant phage tail proteins for capture and detection of the 3 serogroups. The target strains for the inclusivity study and the natural and artificially contaminated foods tested were culturally enriched for 24 h at 41.5°C in buffered peptone water containing cefixime, tellurite and acriflavine. Positive enrichment broths were immuno-purified using immuno-capture before streaking on the appropriate chromogenic or selective plates, to facilitate identification and confirmation.

Results: Inclusivity : the 18 *E. coli* O26, 6 *E. coli* O111 and 18 *E. coli* O157:H7 strains tested were detected with a limit of detection between 5.103 CFU/ml and 5.104 CFU/ml. Exclusivity: no cross reactivity was observed using 30 non-target organisms enriched in buffered peptone water. 300 raw milk cheeses were tested with the new method and a positive result was obtained for approximately 5% of the samples. Confirmations of these samples isolated only *E. coli* O26 serotype. Further molecular studies were performed to identify any virulence factor.

Significance: The new assay is a promising sensitive tool for the simultaneous detection of the three major serogroups *E. coli* O26, O111 and O157:H7 in food products within 24 h.

P2-27 Prevalence of Shiga-toxin Producing *Escherichia coli* (STEC) in Edible By-products of Cattle Using Multiplex Real-time PCR

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are recognized throughout the world as important zoonotic foodborne pathogens that cause bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). They rarely cause disease in animals, and live in the intestines of healthy ruminants, which are recognized as their main natural reservoir, so they can contaminate meat during slaughtering practices.

Purpose: The purposes of this study were to determine the prevalence of STEC and isolate STEC from edible by-products of cattle using multiplex real-time PCR which detects *stx*1 and *stx* genes.

Methods: A total of 82 samples were collected from omasum (n = 32), abomasum (n = 34), and large intestine (n = 16) purchased from a traditional beef market in Seoul, Korea. All samples (50 g each) were homogenized with mEC (450 ml/sample) and incubated at 36°C for 24 h. For presumptive screening, the multiplex real-time PCR was performed to detect shiga toxin 1 and 2 (*stx*1 and *stx*2) in enriched mEC. Positive samples in the presumptive screening were then streaked on

sorbitol MacConkey agar (SMAC). After incubating at 36°C for 24 h, five colonies per each sample from the SMAC were reanalyzed, using the multiplex real-time PCR. The positive colonies in the real-time PCR were streaked on Tryptic Soy Agar with 0.6% yeast extract (TSAYE) and incubated at 36°C for 24 h, followed by biochemical confirmation testing using API 20 E.

Results: In the presumptive screening of enriched mEC, all samples from omasum and large intestine were positive for at least one of the two toxin genes (*stx*1 and *stx*2) by the multiplex real-time PCR method, while 88% (30/34) were positive in abomasums. When five presumptive colonies per samples from the SMAC were reanalyzed using real-time PCR and biochemical confirmation, total STEC prevalence (STEC sample number/Total sample number) rates were 25% (8/32) in omasum, 20% (7/34) in abomasum, 37.5% (6/16) in large intestine and a total of 32 STEC strains were isolated from all samples (11 strains from omasum, 10 strains from abomasum and 11 strains from large intestine).

Significance: This study shows that a high percentage of the edible by-products from cattle were contaminated with STEC, and the multiplex PCR can be used as a confirmatory means of isolating STEC.

P2-28 Reclassification of ATCC® 49444™ from *Staphylococcus aureus* to *Staphyloccus pseudintermedius*

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Introduction: Strain ATCC 49444, currently known as *Staphylococcus aureus*, has been designated as a component strain in both FDA's Bacteriological Analytical Manual and USDA-FSIS's Laboratory Guidebook (MLG 8.06) for the CAMP (Christie-Atkins-Munch-Peterson) test for speciation of *Listeria* isolates. This assay is based on the synergistic hemolytic activity of *L. monocytogenes* that occurs in the presence of beta-lysin producing staphylococci.

Purpose: While conducting authentication tests on this strain, we observed that its genetic and biochemical characteristics were not consistent with its designation as *S. aureus*, but rather *S. intermedius* or the recently described *S. pseudintermedius*, depending on the method used for identification.

Methods: A culture from the original seed lot was subjected to a series of polyphasic tests and compared to closely related Staphylococcal Type cultures as well as commonly used QC strains from the ATCC collection to determine the correct identification of this strain.

Results: Phenotypic analysis of ATCC 49444 using a bioMérieux Vitek 2 Compact identified the strain as *S. intermedius*. Since *S. intermedius* and *S. pseudintermedius* strains cannot be distinguished biochemically, the nucleotide sequences of the 16S rDNA and sodA genes from ATCC 49444 were determined and found to be consistent with other *S. pseudintermedius* strains. Riboprint analysis of ATCC 49444 indicated a low (0.1 to 0.3) similarity index with *S. aureus* or *S. intermedius* strains, but > 0.75 similarity index with *S. pseudintermedius* isolates. A functional comparison of ATCC 49444 with *S. aureus* ATCC 25923 utilizing the CAMP test demonstrated equivalent hemolytic enhancement with *L. monocytogenes*.

Significance: While this study demonstrates that ATCC 49444 is a valid component strain for the CAMP test, it is proposed that ATCC 49444 be reclassified as *S. pseudintermedius* and that users worldwide be alerted to this name change.

P2-29 Evaluation of the TEMPO® STA Method for the Enumeration of *Staphylococcus aureus* in Foods

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Introduction: The TEMPO® STA method was developed for the automated enumeration of *Staphylococcus aureus* in foods. The method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number.

Purpose: As part of the AOAC Research Institute validation process, the alternative method was compared to the FDA Bacteriological Analytical Manual (BAM) method for all foods.

Methods: Fourteen naturally and artificially contaminated foods were tested, including meat, poultry, dairy, seafood, confectionary, and vegetables. Five replicates of three lots for each food were tested, for a total of 210 samples. A 1:10 dilution of each sample was prepared and stomached for 2 min. For each diluted and stomached sample, 1.0 ml of diluted food sample was added to a TEMPO medium vial that had been reconstituted with 3.0 ml of sterile distilled water. The inoculated medium in the vial was then transferred and sealed into the STA card by the automated TEMPO filler. The inoculated cards were incubated for 26 h at 35 ± 1°C. Cards were read using the automated TEMPO reader. Standard method testing was performed as detailed in the BAM.

Results: For the majority of samples tested, there was no significant difference for both the mean log counts and repeatability between the alternative method and the standard method, using a paired *t*-test and *f*-test at the 5% level.

Significance: TEMPO STA provides an automated, accurate method for the enumeration of *Staphylococcus aureus* in foods. In addition, the TEMPO STA offers a considerable time and labor savings when compared to the reference methods tested in this study.

P2-30 Comparison of the 3M™ Tecra™ *Staphylococcus aureus* Visual Immunoassay to the United States Pharmacopeia Standard Method for the Detection of *Staphylococcus aureus* in Raw and Finished Dietary Supplements

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Introduction: Staphylococcus aureus has been reported to be a microbial contaminant in many foods, cosmetic/pharmaceutical products and dietary supplements. Because of the pathogenicity of *S. aureus*, the United States Pharmacopeia (USP) requires its absence in certain non-sterile pharmaceutical raw materials and finished products. There is a need to apply new technologies to conduct the microbiological monitoring of raw and finished products. These technologies need to be rapid, sensitive, accurate, cost effective, and easy to use. The standard microbiological methods require 4 to 5 days for the complete isolation and identification of *S. aureus*.

Purpose: The purpose of this study was to develop a 24 or 48 hour procedure following USP guidelines and testing using the 3M™ Tecra™ *Staphylococcus aureus* Visual Immunoassay, comparing this to the USP method for the detection of *S. aureus* in raw and finished dietary supplements.

Methods: Twelve different raw and finished dietary supplements were artificially contaminated at a low and zero level with *S. aureus* ATCC 6538. Each sample was enriched for 24 ± 2 and 48 ± 2, tested for the presence of *S. aureus* using the 3M™Tecra™ *Staphylococcus aureus* VIA kit, and compared to the Standard USP detection method for *S. aureus*.

Results: The data suggests that an initial 1:10 dilution of each sample that is incubated for 24 ± 2 h, followed by another 1:10 dilution and an additional incubation, for a total of 48 ± 2 h, showed the best recovery of the inoculated samples. Ten of the samples tested showed exact agreement between the 3M™ Tecra™ *Staphylococcus aureus* VIA and the cultural method for the detection of *S. aureus*. Two of the products showed no recovery of the inoculum by either the 3M™ Tecra™ *Staphylococcus aureus* VIA and the cultural method. Both of these products have known antimicrobial properties. Further dilution may be required.

Significance: 3M™ Tecra™ *Staphylococcus aureus* Visual Immunoassay is an easy to use recommended alternative method for the detection of *S. aureus* and meets the USP requirements for detection of *S. aureus* in artificially contaminated dietary supplement products.

P2-31 DSC Comparison of Conventional Culture Method and Real-time PCR for Detection of *Staphylococcus aureus* in Foods

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Introduction: Staphylococcus aureus is one of the most commonly foodborne pathogens, widely distributed on nature and associated to general infection and foodborne outbreaks. Various detection methods have been used for detection of *S. aureus* from contaminated foods. Therefore validation of various detection methods is required in public health.

Purpose: The purpose of this study was to compare the conventional culture method and real-time PCR for detection of *S. aureus* in artificially inoculated foods.

Methods: The bulk food samples (500 g) were artificially inoculated with *S. aureus*. Inoculation levels were 4 and 18 CFU/500 g in sausage and 14 and 10^4 CFU/500 ml in milk and 1200 and 1240 CFU/500 g in vegetable salad to generate partial positive samples. The inoculated food samples were then divided into 20 samples (25 g or ml each), and three negative and one positive control were included in each experiment. All samples were added to mTSB (225 ml/sample) with 10% NaCl and incubated at 36°C for 24 h. After the enrichment, broth cultures were streaked onto a selective medium, Baird Parker Medium with egg yolk tellulite, and incubated at 36°C for 24 h. In addition, 1 ml of broth cultures was collected to be tested by real-time PCR using a commercial kit that detects femA gene. Two suspicious colonies from the selective media were picked up and plated on Tryptic Soy Agar with 0.6% yeast extract (TSAYE) and incubated at 36°C for 24 h followed by coagulase confirmation test.

Results: In sausage, the number of positive samples detected was similar by culture method and with the real-time PCR, 25/40 (62.5%) and 24/40 (60%), respectively. In milk, the culture method and real-time PCR detected *S. aureus* equally well, 27/40 (67.5%) and 25/40 (62.5%), respectively. However, in vegetable salad, the numbers of positive samples detected by conventional culture methods were higher than the real-time PCR, 37/40 (92.5%) and 24/40 (60%), respectively. In addition, negative control resulted in false positive by the culture method, but was defined to negative by real-time PCR.

Significance: This study shows that real-time PCR is a more sensitive and specific detection method, and the culture method requires higher selectivity to exclude false positive result in food samples with high levels of background flora such as in vegetable salad.

P2-32 DSC A *toxR*-based Loop-mediated Isothermal Amplification Assay for Detecting *Vibrio parahaemolyticus*

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Introduction: Vibrio parahaemolyticus is an important foodborne pathogen widely present in warm coastal and estuarine waters and shellfish worldwide. A large number of seafood-associated outbreaks due to *V. parahaemolyticus* have been reported. Sensitive and specific detection methods are needed to better control *V. parahaemolyticus* infections associated with seafood.

Purpose: This study aimed at developing a highly specific and sensitive loop-mediated isothermal amplification (LAMP) assay targeting on the *toxR* gene of *V. parahaemolyticus* and comparing it with a previously described LAMP assay targeting on the *V. parahaemolyticus* thermolabile hemolysin (*tlh*) gene.

Methods: Firstly, a set of four LAMP primers, two outer and two inner, targeting on the *V. parahaemolyticus toxR* gene were designed and optimized. Secondly, the specificity of the assay was evaluated using 18 *V. parahaemolyticus* strains and 32 other strains. To test for the assay sensitivity, serial dilutions of a *V. parahaemolyticus* culture ranging from 1.2×10^6 cell to extinction per reaction were tested. Finally, comparison between the *toxR*-based LAMP and *tlh*-based LAMP was conducted for both sensitivity and specificity.

Results: Both *toxR*- and *tlh*-based LAMP assays were able to specifically detect the 18 *Vibrio parahaemolyticus* strains with no false positives for other strains. The detection limit observed was 1.2×10^2 cells for the *toxR*-based LAMP, compared with 1.2 cell for the *tlh*-based LAMP.

Significance: The *toxR*-based LAMP assay developed in this study for detecting *V. parahaemolyticus* was sensitive and specific, and holds great potential for future field applications.

Poster

P2-33 Rapid Detection of *Vibrio vulnificus* in Oysters with Immunomagnetic Separation Real-time PCR Assay

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Introduction: Vibrio vulnificus is considered one of the deadliest among all human pathogenic vibrios, with about 50% mortality rate. The majority of *V. vulnificus* infections in the United States are due to consumption of raw molluscan shellfish. The current conventional bacteriological detection methods are very labor intensive and time consuming. Real-time PCR (q-PCR) in combination with immunomagnetic separation (IMS) could be a rapid approach to detect *V. vulnificus* in complex environmental samples such as oyster meat.

Purpose: The aim of this study was to develop and optimize an IMS q-PCR protocol that uses anti-H antibody to rapidly concentrate and quantify *V. vulnificus* from oyster homogenate.

Methods: Monoclonal antibodies (MAbs) were used to prepare two different sets of immunomagnetic separation reagents at a concentration of 5 µg/10^7 immunomagnetic beads (IMB). Separation of *V. vulnificus* cells from the spiked oyster homogenate was achieved by incubating 500 µl of oyster homogenate with 10^7 MAb coated IMB on a shaker at 25°C for 30 min. The eluted *V. vulnificus* cells were then lysed by heating and assayed by q-PCR, using primers and probes derived from the *V. vulnificus* structural gene for cytolysin, vvhA.

Results: IMB coated with MAbs 3-D-10 and 8-D-4 when coupled with qPCR were able to identify 42% and 37% *V. vulnificus* cells, respectively, in the oyster homogenate. The minimum detection limits were 1.7×10^3 and 2.3×10^3 cells/ml of oyster homogenate respectively for the above stated antibodies.

Significance: The IMS q-PCR can be employed to identify and quantify *V. vulnificus* from oysters in < 3 h, eliminating an enrichment step and separating target organisms from PCR inhibitors present in oyster tissue.

P2-34 Rapid Capture and Detection of Model Viruses from Large Volumes of Water DSC

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Introduction: Foodborne viral infections caused by the noroviruses and Hepatitis A virus are mainly the result of viral contamination during food production due to infected food handlers, or food contact with sewage sludge or polluted water. Current methods to rapidly assess the presence of foodborne viruses in water used for food production are limited. Consequently, there is a need for development of rapid and sensitive methods for the capture and detection of foodborne viruses in large volumes (10 liters or more) of water.

Purpose: The objective of this study was to evaluate an integrated sampling and detection method to rapidly assay for foodborne viruses in large volumes of water.

Methods: FRNA bacteriophages MS2, GA, SP and Qβ were used in all experiments. These phages were chosen based on their differing isoelectric points, and their acceptance as surrogates of foodborne viruses in the scientific literature. Each phage was individually seeded into 10 liter volumes of tap water at a concentration of 10^0, 10^1, 10^2 and 10^3 PFU mL^{-1}. One and a half grams of anionic exchange resin (Amberlite® IRA 900) was added to each water sample and kept in suspension by continuous agitation. Aliquots of water were withdrawn at 0 and 60 minute time points and assayed for phage via the double layer plaque assay. Virions bound to the resin beads were detected by isolating viral RNA directly off of the beads, followed by reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR using a SYBR Green assay.

Results: The resin captured phages MS2, Qβ and SP with high efficiency (ranging from 86% (phage Qβ, 10^2 PFU mL^{-1}) to 100% (phages SP, Qβ, and MS2 (10^0 PFU mL^{-1}). The capture efficiency of phage GA was lower and ranged from 55% (10^0 PFU mL^{-1}) to 89% (10^2 PFU mL^{-1}). When RT-PCR and agarose gel electrophoresis was used to detect the phages the detection limits were 2.0×10^2 PFU mL^{-1} (SP), 4.0×10^1 PFU mL^{-1} (MS2), 5.5×10^0 PFU mL^{-1} (GA) and 5.7×10^2 PFU mL^{-1} (Qβ). Real-time RT-PCR detection limits were 1.0×10^1 PFU mL^{-1} (SP), 3.0×10^0 PFU mL^{-1} (MS2), 5.5×10^0 PFU mL^{-1} (GA) and 1.0×10^0 PFU mL^{-1} (Qβ).

Significance: These results suggest that anion exchange capture and real-time RT-PCR can be used as a rapid and sensitive assay for detection of foodborne viruses. This method would be especially useful to test water used during the production of foods that are consumed raw, such as produce and certain seafoods.

P2-35 Evaluation of Repetitive Extragenic Palindromic Sequence-based PCR Typing of *Bacillus* Species

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Introduction: The application of molecular subtyping methods to bacterial foodborne pathogens provides the tools to detect and track outbreaks and contamination sources throughout the food system. Pulsed-field gel electrophoresis is often considered the "gold standard" for molecular subtyping, as the method offers a high level of discrimination; however, the drawback is that it is a time-consuming process that requires a high level of skill to perform. Automated subtyping methods for ribotyping and repetitive extragenic palindromic sequence-based PCR (rep-PCR) have emerged as approaches to rapidly discriminate bacterial strains.

Purpose: The purpose of this study was to assess the utility of the DiversiLab System™, an automated rep-PCR-based typing system, to effectively differentiate species and strains of the genus *Bacillus*.

Methods: A total of 79 *Bacillus* strains, representing 19 species, from the Silliker Laboratories Research Culture Collection were used in this study. Isolate DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit, and DNA was amplified using the DiversiLab *Bacillus* strain typing kit. Rep-PCR products were separated and detected using microfluidics chips and the Bioanalyzer platform. Sample fingerprints were analyzed using the web-based DiversiLab software.

Results: Rep-PCR fingerprints were generated for every sample. Distinct fingerprint patterns were generated for strains within a species-specific cluster. For example, unique fingerprints were generated for several strains of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*, and the majority of the strains for a particular species clustered together. In addition, strains representing the *B. cereus* group, including *B. cereus*, *B. thuringiensis*, and *B. mycoides*, clustered together in the analysis.

Significance: These data suggest that automated rep-PCR typing is an effective tool for discrimination of *Bacillus* species and strains. Potential applications for this technology include microbial source or spread tracking (e.g., to compare strains isolated at different locations within a food processing plant) or differentiation of bacterial strains within a single food product.

P2-36 New Simplified Short Protocol for Rapid Detection of *Cronobacter* spp. in Powdered Infant Formula, Ingredients and Environmental Samples

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Introduction: Contaminated Powdered Infant Formula (PIF) has been identified as the source of *Cronobacter* spp. (*Enterobacter sakazakii*) ingestion by neonates. The European Commission and FDA have required the absence of *Cronobacter* in PIF intended for consumption by infants less than 6 months of age. Furthermore, PIF manufacturers and their suppliers also test environmental samples and raw materials to improve risk management. However, it has now been established that some strains of *Cronobacter* do not grow well in the enrichment broths proposed in ISO and BAM Standards, and this could lead to false-negative results.

Purpose: The aim of the present study was to evaluate a new one-step enrichment protocol for the screening of *Cronobacter* spp. from powdered infant formula, ingredients, baby food and environmental samples. This protocol consists in a combined pre-enrichment/enrichment broth (*Cronobacter* Enrichment Broth, bioMérieux Marcy l'Etoile, France) used in conjunction with a selective-differential agar ChromID Sakazakii (bioMérieux Marcy l'Etoile, France) to facilitate a shortened two-day cultural method for the detection of *Cronobacter*.

Methods: The *Cronobacter* Enrichment Broth (CEB) was evaluated using naturally contaminated environmental samples as well as PIF, raw material and baby food samples spiked with low concentrations of lyophilized *Cronobacter* strains. The isolation of strains was compared in parallel with the current ISO/TS 22964 and a recently proposed differential screening broth (CSB) method (Iversen et al., 2008, AEM vol 74 p. 2550-53) for the detection of *Cronobacter*.

Results: The use of CEB enrichment followed by plating on ChromID Sakazakii™ resulted in greater recovery than when using the other broths for recovery of *Cronobacter* from PIF, ingredients and naturally contaminated environmental samples. All of the Cronobacter strains were recovered from PIF using the CEB whereas one strain was not detected using either the ISO standard and the CSB method. For baby food samples, twice as many *Cronobacter* colonies were isolated using CEB enrichment followed by streaking on ChromID Sakazakii™ than by the other isolation methods used.

Significance: This study found CEB to be significantly better at recovering *Cronobacter* spp. from PIF, ingredients, baby food and environmental samples than the other enrichment media tested. Overall, the use of CEB with a differential plating media such as ChromID Sakazakii permits a faster release (40 to 48 h) of products (e.g., baby foods and ingredients) and facilitates the rapid testing of environmental samples allowing better risk management.

P2-37 Isolation of Yellow-pigmented Enterobacteriaceae from Japanese Style Box-lunch and Misidentification as *Enterobacter sakazakii* by Several Identification Kits

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Introduction: Enterobacter sakazakii, belonging to the family Enterobacteriaceae, causes several opportunistic infections, such as meningitis and bacteremia, especially in neonates and infants. *E. sakazakii* has been isolated from powdered infant formula, cheese, meat, vegetables, fruit powder and environment.

Purpose: Although several isolation procedures were reported for *E. sakazakii*, only a few identification methods have been established. Several studies have recommended using identification kits. In this study we evaluated three identification kits.

Methods: Samples were homogenized and spread on a RAPID' E. coli 2 agar (Bio-Rad). Blue-green colonies on RAPID' E. coli 2 were picked up and streaked on plate count agar (PCA). Yellow-pigmented colonies on a PCA were isolated for further experiments. The isolates were characterized by using API 20E (bioMérieux), BBL crystal E/NF (Becton Dickinson) and ID test EB20 (Nissui). A phylogenetic study of the isolates was based on 16S rRNA gene sequence analysis.

Results: Five yellow-pigmented isolates were obtained from sliced cabbage in three samples, all of which were fermentative gram-negative oxidase-negative rods showing α-glucosidase activity. Four out of the five isolates were identified as *E. sakazakii* with at least one of the three kits. However, all of five isolates exhibited the 16S rRNA gene sequences highly homologous to the bacteria other than *E. sakazakii*. According to the results of the phylogenetic analysis, the four isolates consisted two *Pantoea agglomerans*, one *Escherichia hermannii* and one *Escherichia* sp.

Significance: Several studies have reported that the API 20E and α-glucosidase test were useful to identify *E. sakazakii*. The current US Food and Drug Administration method to detect *E. sakazakii* includes API 20E for a species confirmation. However, the present study suggests that several members in Enterobacteriaceae could be misidentified as *E. sakazakii* by these methods, and that 16S rDNA sequencing is helpful for the identification.

Poster

P2-38 DNA Aptamers with Binding Specificity for *Campylobacter jejuni*: Application to Pre-analytical Sample Processing

DSC

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Introduction: Antibodies are widely used for pathogen concentration and purification prior to detection. Alternative ligands, including nucleic acid aptamers, have the potential to provide binding specificity equivalent to antibodies but with advantages such as low cost, ease of manufacture, and improved stability.

Purpose: To identify DNA aptamers demonstrating binding specificity to *Campylobacter jejuni*, and to apply these to pre-analytical sample processing prior to PCR-based detection.

Methods: To identify candidate aptamers, a whole cell-SELEX (Systematic Evolution of Ligands by EXponential enrichment) method was applied to a combinatorial library of FAM-labeled single stranded (ss) DNA molecules. A total of ten SELEX rounds were applied, using *C. jejuni* strain A9a as the target. Periodically, binding was confirmed using flow cytometry and the aptamers demonstrating peak fluorescence were sorted. Selectivity of the aptamers was further enhanced using counter-SELEX methods. The enriched aptamers were cloned, sequenced and analyzed for binding characteristics.

Results: Nine candidate FAM-labeled aptamer sequences with high binding specificity to *C. jejuni* were identified upon challenge with 10^8 to 10^9 *C. jejuni* (A9a) cells. Aptamer ONS-23 showed the highest binding affinity [dissociation constant (K_d value) of 292.8 ± 5.3 nM], with 47 ± 6% of cells fluorescent (bound) at a 1.48 µM aptamer concentration. Aptamer inclusivity was demonstrated by similarly high binding efficiencies (ranging from 25 to 36%) of ONS-23 for different *C. jejuni* strains including ATCC-33560, ATCC-33291, strain 2083, and strain A14a. Binding specificity was confirmed by challenging aptamer ONS-23 with other foodborne bacteria including *Bacillus cereus* strain T, *E. coli* O157: H7, and *L. monocytogenes* ATCC 19115, for which binding efficiencies never exceeded 5%.

Significance: This project demonstrates that DNA aptamers can be selected using a whole cell-SELEX method, with resulting aptamers demonstrating high binding affinity and specificity. Aptamers are currently being evaluated for their ability to capture *C. jejuni* from complex sample matrices for subsequent detection by PCR.

P2-39 Comparison of Real-time PCR and Conventional Culture Method for Detection of *Campylobacter jejuni* in Ground Beef and Vegetable Salad

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Introduction: Campylobacter jejuni (*C. jejuni*) is the leading cause of foodborne gastroenteritis worldwide. Rapid and effective methods have been developed, as the standard culture method requires up to 5 days and is not suitable for routine testing of large numbers of samples. Real-time PCR was evaluated for the timesaving and sensitive detection of *C. jejuni* in food samples using molecular beacon probes.

Purpose: The purpose of this study was to compare the performance of real-time PCR and culture method for the detection of *C. jejuni* in ground beef and vegetable salad.

Methods: Performances of two detection methods (real-time PCR and FDA/BAM reference method) were evaluated for recovery of *C. jejuni* in artificially contaminated food samples. Five hundred grams of samples (ground beef and vegetable salad) were artificially contaminated with various concentrations to generate partial positive, then divided into 20 samples (25g each). Samples were incubated in Bolton broth at 37°C (pre-enrichment for 4 h) and 42°C (enrichment for 44 h), then streaked onto the mCCD agar incubating under microaerobic conditions at 42°C for 48 h, followed by a confirmation test using API Campy. In parallel, real-time PCR was performed using primers and probes targeting the open reading frame (ORF) C sequence specific for *C. jejuni* after enrichment in Bolton broth.

Results: In ground beef, real-time PCR detected the same number or more positives than the culture method, but there was no significant statistical difference (real-time PCR: 65/80, culture method: 59/80, $P = 0.3440$). In vegetable salad, in contrast, the number of positive samples by real-time PCR was much more than with the culture method. In addition, there was an extremely significant statistical difference (real-time PCR: 37/80, culture method: 1/80, $P < 0.0001$) between the two methods.

Significance: Real-time PCR provides more reliable detection of *C. jejuni* than the conventional culture method with fewer steps, especially for foods with high numbers of background flora, like vegetable salad.

P2-40 Immunomagnetic Concentration and Real-time RT-PCR Detection of Norovirus

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Introduction: Norovirus (NoV) is the major agent of acute foodborne gastroenteritis in human worldwide. The concentration of viral particles found in food samples is very low but still sufficient to cause infections in humans and corresponds to the minimal infectious dose. Therefore, the concentration of viral particles prior to detection is necessary to achieve an accurate and sensitive detection of these viruses in clinical, food and environmental samples.

Purpose: The objective of this study was to develop and evaluate the efficacy of an immunomagnetic concentration method combined to real-time RT-PCR for the sensitive and specific detection of NoV.

Methods: Polyclonal antibodies have been produced, using a synthetic peptide of the viral capsid protein as an antigen. The antibodies obtained were purified and immobilized on magnetic beads by covalent link. The magnetic beads capturing viral particles were lysed and submitted to amplification by TaqMan RT-PCR. The concentration and detection approach has been applied to different spiked food samples.

Results: Specific polyclonal antibodies have been successfully produced against NoV and were purified by affinity using protein A/G column. A concentration range of 10-fold from the initial viral count was obtained, using the immunomagnetic capture system. The detection limit of the concentration and detection approach was 0.001 RT-PCRU.

Significance: The development and application of this strategy contribute to a better concentration and detection of NoV in different samples.

P2-41 Rapid Automated Method for the Detection of Yeast and Molds in Cultured Dairy Products

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Introduction: In cultured dairy products, yeast and mold contamination is the most usual cause of spoilage. Such spoilage can be very costly for dairy manufacturers if prematurely spoiled products reach the marketplace. Therefore, yeast and mold levels need to fall within acceptable limits prior to the food leaving the factory. Consequently, reducing the yeast and mold test time from the traditional 5 days to 48 h is very desirable to reduce product holding time. BioLumix has developed a new optical system for rapid and automated detection of yeast and molds in a variety of food products.

Purpose: To evaluate the new BioLumix system and technology for the detection of yeast and molds in cultured dairy products, with regard to speed to results, sensitivity and specificity.

Methods: The BioLumix system detects optical changes in an embedded optical sensor for the detection of CO_2, located at the bottom of the test vial. Yeast and mold growth in the medium above the sensor results in CO_2 production and changes in the sensor's color. The new ready to use yeast and mold vials were compared to the plate count methodology in its ability to detect yeast and molds, if present, in cultured dairy products.

Results: Various dairy products (yogurt, sour cream, cream cheese, cottage cheese, and butter), clean, naturally contaminated or inoculated with different yeast or mold strains at levels of 10 to 10,000 cells/g, were tested. Over 100 combinations of yeast or mold and products were evaluated. All samples that contained yeast or mold were detected by the CO_2 sensor, while none of the un-inoculated samples were detected. Therefore, the system clearly distinguished between clean and contaminated samples. Certain cultured dairy products required the addition of antibiotics to the vial to prevent drift in the curves due to starter culture activity. Most yeasts were detected within 24 h, while molds required 48 h to be detected.

Significance: The data suggests that the new method is useful for determining yeasts and molds in cultured dairy products. It offers a significant reduction in time to results, product holding time and hands-on labor because of its automation and simplicity of use.

P2-42 Comparison of BACARA® Agar, a New Chromogenic Medium, and MYP Agar for the Enumeration of *Bacillus cereus* in Food Samples

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Introduction: The *Bacillus cereus* group includes species such as *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis* and *B. anthracis*, which are well known as human foodborne pathogens. *B. cereus* is the second most commonly involved pathogen in outbreaks caused by bacterial toxins in Europe.

Purpose: The aim of this study was to evaluate the performances of BACARA®, a new chromogenic medium, versus the MYP agar (ISO Mandatory Medium) for the direct enumeration of *B. cereus* in food.

Methods: Inclusivity tests with 100 *B. cereus* group strains and exclusivity tests with 70 non *B. cereus* strains were performed on both BACARA® and MYP. The assessment of the sensitivity, selectivity and ease of counting with the BACARA® method, compared to the ISO7932 method, was carried out on a total of 80 food samples as a second step. The vast majority of those samples were naturally contaminated. Each sample was diluted in a 1/10 Buffered Peptone Water solution, and 0.1 ml of the serial dilutions were inoculated on MYP and BACARA® agar. The MYP and BACARA® agars were incubated at 30°C and 37°C, respectively.

Results: Exclusivity data demonstrated a much better selectivity of the BACARA® agar compared to the MYP agar. Twenty-seven of non-*Bacillus cereus* strains grew on MYP whereas they were all inhibited on BACARA® agar. Although most of these strains didn't have a typical aspect on MYP, they were sources of misinterpretation on high contaminated samples due to an interfering flora on the Petri Dishes. On the other hand, the combination of a specific nutrient base, a strong selectivity and a chromogenic mixture utilised in BACARA® agar allowed obtaining very large and easy to count colonies. For both naturally and artificially contaminated samples, BACARA agar performed well for the enumeration of *B. cereus* compared to the ISO7932 reference method.

Significance: The BACARA® method is a reliable alternative method for the enumeration of *B. cereus* in food. The high level of selectivity and the specific enzymatic detection of typical colonies on this medium can improve interpretation compared to results with the classical MYP agar. BACARA® will help the end-user save time and avoid the necessity for any confirmation protocol.

P2-43 Comparison Study to Demonstrate the Equivalence of the SimPlate Total *Campylobacter*-CI Method to the Reference Culture Method for the Enumeration of Total *Campylobacter jejuni* and *Campylobacter coli* in Food

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Introduction: The SimPlate for Campylobacter Color Indicator (C-CI) method allows for the quantitation of total *Campylobacter jejuni* and *Campylobacter coli* in poultry meat and poultry meat rinses after 48 to 52 h of incubation in a microaerophilic environment.

Purpose: A study was undertaken to compare the SimPlate C-CI method to the reference culture method for the quantitation of total *Campylobacter jejuni* and *Campylobacter coli*.

Methods: Target *Campylobacter* and non-target microorganisms were tested for inclusivity and exclusivity by the SimPlate method. 37 strains of *C. jejuni* and *C. coli* were enriched in Bolton broth, diluted and plated onto SimPlate devices and 3 selective agar plates (Abeyta-Hunt-Bark (AHB) agar, Campy CEFEX agar and Line agar). Finally, a field trial comparison of the performance of the SimPlate C-CI method to the Campy CEFEX method was performed. Lab personnel at 3 poultry processing plants analyzed 168 BPW carcass rinse samples with both methods.

Results: There was good correlation for the quantitation of *Campylobacter* from all three plating methods to the SimPlate method; only 2 strains for AHB and 1 strain for Line agar demonstrated greater than 0.5 log difference between both methods. For exclusivity, the C-CI method detected none of the 27 non-target organisms tested. Regression analysis of the results from the field trial comparison showed a correlation of 0.96.

Significance: These results indicate that the SimPlate C-CI method and the reference culture method are comparable for enumeration of *Campylobacter jejuni* and *Campylobacter coli* in poultry meat and poultry meat rinses.

P2-44 Comparison of Assurance GDS for *Escherichia coli* O157:H7 Enrichment Ratios with Composite Beef Samples

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Introduction: A study was conducted to determine the efficacy of detecting low contamination levels of *E. coli* O157:H7 in 375 g beef trim samples at three sample to media ratios, using Assurance GDS™ for *E. coli* O157:H7.

Purpose: To evaluate the effectiveness of enriching 375 g beef trim samples in mEHEC media at a sample:media ratio of 1:4.2, 1:5 and 1:10 for detection of *E. coli* O157:H7 with Assurance GDS™ for *E. coli* O157:H7.

Methods: 240 test portions of 375 g beef trim were artificially contaminated with low levels of cold-stressed *E. coli* O157:H7. The beef trim received an inoculation of stationary phase cells sufficient to achieve a contamination level of 1.2 to 1.9 CFU/375g. A total of 80 test portions were analyzed for each of the three media ratios. Test portions were run at ratio 1:4.2 (375 g in 1.2 L mEHEC media), 1:5 (375 g in 1.5 L mEHEC media) and 1:10 (375 g in 3.375 L mEHEC media). Test portions were analyzed by Assurance GDS for *E. coli* O157:H7 following 8, 10 and 12 h of incubation in mEHEC media. All test portions were culturally confirmed via a modified USDA method.

Results: All three enrichment ratios tested showed a sensitivy level of 98% or greater at 8 to 12 h of incubation at low inoculation levels.

Significance: Low levels of *E. coli* O157:H7 can be accurately detected in 375 g beef trim samples using Assurance GDS™ for *E. coli* O157:H7, using three sample to media ratios at 8 to 12 h of enrichment.

P2-45 Detection of Shiga Toxin-producing *Escherichia coli* (STEC) with the Assurance GDS for STEC assay

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Introduction: Recent reports of illnesses caused by foodborne non-O157:H7 Shiga Toxin-producing *Escherichia coli* (STEC) have led to increased awareness of their threat to public safety.

Purpose: A rapid screening assay has been developed to detect *E. coli* STEC isolates with the following O-serotypes: O26, O45, O103, O111, O121, O145.

Methods: Immunomagnetic beads are employed to specifically isolate and concentrate bacteria that express these O-antigens during a sample preparation step. DNA from the samples is then amplified and identified using primers and probes directed against conserved, specific, virulence-associated DNA sequence targets in these bacteria.

Results: The assay was able to detect 30/31 *E. coli* strains that expressed one of the O-antigens in question. The one undetected strain did not contain either the *stx*1 or *stx*2 gene and is not considered a STEC. An additional 40 bacteria, including 15 E. coli strains that express different O-antigens, were not detected.

Significance: The data show that the combination of an immunomagnetic sample preparation step and a specific DNA amplification-detection step yield a screening assay specific and sensitive for the top 6 *E. coli* STEC strains known to cause human disease.

P2-46 Development of a Scorpion™ Probe-based Real-time PCR Assay for Genus *Salmonella*

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Introduction: The use of PCR-based methods for *Salmonella* spp. detection and monitoring have shown tremendous growth in recent years. One commonly used commercial method, the BAX® system, uses end-point PCR based on melting curve analysis. Although this method features excellent performance characteristics for sensitivity and specificity, it can require nearly 3.5 h to complete the cycling and melt curve analysis.

Purpose: The purpose of this study was to evaluate the use of probe-based Scorpion™ technology with existing primer sequences to develop a faster real-time PCR assay that would maintain performance identical or superior to the current end-point PCR assay. The use of probe detection allows for much more rapid cycling (< 1 h) and eliminates the need for a melt curve analysis.

Methods: Studies comparing the sensitivity and inclusivity of the new real-time assay with the current commercial PCR assay were conducted, using both purified DNA and select *Salmonella* spp.

Results: Results using liquid real-time PCR reagents versus the tableted commercial PCR kit reagents showed equivalent sensitivity using both DNA (5 to 50 fg) and cells (~10^4 CFU/ml). Inclusivity using a small panel of 48 diverse *Salmonella* spp. was also identical with both assays, showing 100% detection of the strains tested.

Significance: These results demonstrate the feasibility of developing a novel real-time PCR assay for *Salmonella* spp. that allows for cycling and detection in less than one hour, with the same performance characteristics of an existing well-characterized, commercial assay.

P2-47 Monitoring Cryptic Growth of *Escherichia coli* at 6°C by Transfer to 37°C

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Introduction: Escherichia coli is recognized as an indicator for the behavior of enteric pathogens such as *E. coli* O157 and *Salmonella*. Keeping chilled foods at temperatures below the minimum for sustained growth of *E. coli* (7°C) is then regarded as safe practice. Formation of filaments by *E. coli* and related pathogens at < 7°C may result in underestimation of microbiological risks if filaments yield multiple daughter cells upon temperature increase.

Purpose: To determine increases in colony counts when chilled *E. coli* are exposed to warm temperatures.

Methods: Log phase *E. coli* ATTC 23739 was incubated at 6°C for 10 days. Each day samples were incubated at 37°C for 2 h. Filament formation at 6°C was monitored by photomicroscopy, while growth at 6 and 37°C was monitored by enumeration of CFU and absorbance at 600 nm (A600).

Results: At 6°C absorbance values increased by 0.015 log unit/day to day 10 and filaments (> 4 µm) were formed, while the numbers of CFU increased by 0.1 log units/day until day 3 and then remained constant. At zero time, CFU in samples incubated at 37°C increased by 1 log unit, but increases at later times ranged up to 1.5 log unit at 4 day intervals.

Significance: Chilled, filamentous *E. coli* divide into multiple daughter cells when shifted to higher temperatures. Enumeration of CFU for mesophilic organisms in chilled foods may underestimate the numbers of organisms to which consumers would be effectively exposed.

P2-48 Development of a New ComBase-derived Database of Microbial Responses to Food Environments: Microbial Responses Viewer (MRV)

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Introduction: In order to establish food processing and distribution guidelines, food processors are required to employ processing conditions that prevent microbial growth. Exploring targeted bacterial growth or no growth conditions has been recognized as an important component of ensuring food safety. ComBase, a large database of microbial responses to food environments, has attracted the attention of many researchers and food processors. Although ComBase contains a vast amount of data, it is not easy to obtain desired information from the retrieved data.

Purpose: The aim of this study was to develop a web-based database, MRV (Microbial Responses Viewer: http://cbnfri.dc.affrc.go.jp/), consisting of bacterial growth/no growth data classified from ComBase, using specific criteria. MRV can retrieve bacterial growth/no growth data defined under specified environmental conditions of temperature, pH, and a_w. In addition, MRV simultaneously retrieves growth rate data produced under specified environmental conditions.

Methods: The response was defined as representing "growth" if a significant increase in bacterial concentration (> 1.0 log) was observed in the ComBase data. Alternatively, "growth" was defined as a positive value of the specific growth rate. Furthermore, the specific growth rate of each microorganism was modelled as a function of temperature, pH, and water activity (a_w) by use of a Poisson log-linear model, which is a family of generalized linear models (GLM).

Results: The growth/no growth data of nineteen different microorganisms were extracted from all the data in ComBase comprising 29 kinds of microorganism. For 16 of the 19 microorganisms, the specific growth/death rate was successfully modelled as a function of temperature, pH, and a_w using GLM. The specific growth rate was illustrated using a two-dimensional contour plot with growth/no growth data. MRV provides information concerning growth/no growth boundary conditions and the specific growth rates of specified microorganisms.

Significance: This innovative database facilitates the retrieval of growth/no growth data for various kinds of bacteria and will contribute to ensuring microbiological food safety. Using MRV, food processors can easily find the appropriate food design and processing conditions. This database will contribute to the efficient and safe production and distribution of processed foods.

P2-49 Generic Modeling Approach for Quantitative Microbial Risk Assessment

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Introduction: Quantitative microbial risk assessment (QMRA), a holistic approach to food safety, is most often accomplished using Monte Carlo simulation methods to combine existing knowledge and data into a prediction of risk. The prediction of risk is relative rather than absolute because of knowledge, data and model uncertainty. However, through the process of scenario analysis, relative risk can be assessed and used to help inform risk management decisions.

Purpose: A generic simulation model for QMRA was developed and scenario analysis was then used to demonstrate how the model can be used to make food safety decisions.

Methods: A processing plant exit to table model was constructed in an Excel spreadsheet and was simulated using @ Risk. Module I for hazard identification and exposure assessment consisted of five process steps and associated pathogen events. Module II for hazard characterization and risk characterization included a novel dose-response model that predicted response dose as a function of normal and high risk classes for hazard, food and host factors. Two scenarios were simulated, one for a high incidence (25%) of hazard contamination at packaging (plant A) and one for a lower level (10%) of hazard contamination at packaging (plant B).

Results: Simulation results indicated that the median number of responses or cases per 100,000 food units was 3 (range 0 to 11) for plant A and 7.5 (range 1 to 14) for plant B. Higher cross-contamination during meal preparation and higher incidence of high-risk hosts and hazards in the distribution channel for plant B accounted for these unexpected results of greater risk from the plant producing the less contaminated product.

Significance: This study demonstrates the importance of considering post-process risk factors when assessing safety of food at the processing plant.

P2-50 Risk Ranking Tool for Prioritizing Commodity and Pathogen Combinations for Risk Assessment of Fresh Produce

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Introduction: Outbreaks associated with fresh produce have increased in the past decade. There is currently no transparent, data-driven, customizable ranking system that can be used to rapidly prioritize pathogen-commodity pairs for more rigorous risk assessment modeling efforts.

Purpose: To develop a semi-quantitative risk ranking software tool to prioritize and rank pathogen-commodity combinations, based on explicit data-driven risk criteria.

Methods: To identify candidate pathogen-commodity pairs, a database was created that included all reports of fresh produce-associated outbreaks compiled by the CDC (1996 to 2006). Additional information was sought from peer-reviewed literature and publicly accessible databases. Nine risk criteria were developed across four primary dimensions of risk: (1) strength of epidemiological association between pathogen and commodity; (2) severity of disease; (3) pathogen characteristics that influence disease outcome; and (4) commodity characteristics that influence pathogen prevalence, behavior, and likelihood of exposure. For each risk criterion, narrative descriptions were developed and quantified for scoring purposes, and available data were used to score each criterion. User-specified weights were assigned to each criterion based on the user's judgment regarding the relative contribution to risk. The overall risk score for any one pathogen-commodity pair is the summation of the criteria scores multiplied by the respective criteria weights.

Results: A total of 51 pathogen-produce commodity pairs were included in the risk ranking. Ranking scores ranged from a low of 13 to a high of 155. Scenario analyses were performed to explore the impact of user-defined weights on the ranking results. Within the range of weights that were considered, enterohemorrhagic *E. coli* and leafy greens consistently ranked first, followed by *Salmonella* spp. and tomatoes and *Salmonella* spp. and leafy greens.

Significance: The risk ranking tool provides a systematic, transparent, and customizable tool with which to prioritize pathogen-commodity pairs for more rigorous risk assessment modeling efforts.

P2-51 Validation of Growth Predictive Model for *Staphylococcus aureus* in Ready-to-Eat Foods
DSC

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Introduction: Recently, consumption of Ready-to-Eat food (RTE) has increased because of customers' needs and preferences. However, the safety of RTE foods must be assured in the retail market because these foods are consumed without heating. Especially, *Staphylococcus aureus* (*S. aureus*) is recognized as a major pathogenic contaminent of RTE foods such as Kimbab (rice rolled in laver) and sandwiches in Korea.

Purpose: The objective of this study was to validate growth predictive models developed for *S. aureus* in Kimbab and sandwiches as a function of temperature.

Methods: S. aureus producing enterotoxin A, C, or D were used. Commercially prepared Kimbab and sandwich were artificially inoculated with a cocktail of three *S. aureus* strains at an initial concentration of 3 log CFU/g and stored at 8, 13, 18, 23 or 28°C, which was not used for model development. The growth kinetic parameters at each temperature were determined by the Gompertz equation, using GraphPad Prism. Specific growth rate (SGR) and lag time (LT) values were further used for the Square-root model and Davey model, respectively, as a function of temperature. Model performance based on the bias (Bf) and accuracy (Af) factors were calculated.

Results: In the primary model, SGR and LT were mainly affected by temperature in Kimbab and sandwich. For Kimbab, Bf and Af were 1.07 and 1.12; respectively, for SGR, 0.99 and 1.23 for LT. For sandwich, Bf and Af were 1.06 and 1.23, respectively, for SGR, 1.00 and 1.18 for LT, indicating that the induced secondary model shows suitable performance in predicting the growth of *S. aureus* in both Kimbab and sandwich.

Significance: These validated models will be used in the development of tertiary model for Kimbab and sandwich industry to quantify the effect of temperature on the growth of *S. aureus*.

P2-52 Predictive Modeling for Growth of *Staphylococcus aureus* on Steamed Soybean Sprouts with Seasoning in School Foodservice Operations

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Introduction: Due to the increased popularity of restaurant food and catered meals, the frequency of microorganism-related, foodborne outbreaks has climbed dramatically in recent years. Korean cooked vegetables have a mixing process with hands after heating and are more susceptible to contamination by *S. aureus* than many other types of vegetable preparations. The guidelines for food safety management in school foodservice of Korea must be re-established, taking into consideration a quantitative analysis of traditional Korean food culture.

Purpose: This research develops a predictive model that can be used to evaluate microbiological safety of steamed soybean sprouts with seasoning. The study provides basic data on the process of acquiring microbiological information regarding the quality of foods prepared with complex cooking processes.

Methods: Microbiological analysis was performed on times from 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 12, 24, 48, and 72 hours at four storage temperatures (5, 15, 25, and 35°C). Both the model of Gompertz and Baranyi and Roberts were fitted to the logarithm of the viable cell concentration. The second stage of modeling concentrated on describing the variation of the parameters of the growth curve as a function of growth condition using response surface model.

Results: In primary model, R2 values were 0.9397 (5°C), 0.9334 (15°C), 0.9640 (25°C), and 0.9914 (35°C) and Root Mean Square Error were 0.07 at 5°C; 0.10 at 15°C; 0.11 at 25°C; and 0.08 at 35°C. On the basis of RS models obtained, temperature was the most important factor ($P < 0.05$). The validation of the overall model was quantified by determining, the adjusted R2(89.31%), the RMSE (0.14) and, the Bf (0.9950) and Af (1.065).

Significance: This study is the initial trial of implementing predictive microbiology in Korean foodservice operations. The results in the present study showed that the developed predictive model was capable of predicting *S. aureus* growth under isothermal temperature storage conditions, giving good predictions of the viability of steamed vegetables with seasoning at various school foodservice operations.

P2-53 Simulation of Factors Important in Norovirus Transmission in Foodservice Systems
DSC

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Introduction: Norovirus (NoV) is currently regarded as the leading cause of gastroenteritis in the United States. The Centers for Disease Control and Prevention (CDC) estimate that 23 million cases of acute gastroenteritis are caused by NoV annually and 39% of all recorded NoV outbreaks occur in restaurant settings. In a foodservice system, foodhandler-hygiene can play an important role in virus spread and cross-contamination.

Purpose: The purpose of this study was to build a simulation model that mimics the complex interactions involved in NoV transmission, which may take place in a foodservice system and to use the model to study interventions that might reduce risk.

Methods: Data from the peer-reviewed literature were collected and used to build the model. Due to the limited availability of published data, our model focused primarily on quantifying the effects of specific steps on NoV transmission including (1) transmission between food, hands and other possible sources of contamination, (2) virus survival on different surfaces and (3) the effect of different hand treatments on virus reduction. The model was built with the discrete-event simulation package, Arena® (Rockwell International), and used to simulate foodservice worker movement, as well as virus transfer and survival.

Results: The model predicts that with no handwashing, all the exposed individuals will become ill. When handwashing was used as an intervention, the predicted fraction that became ill fell by 25 or 30%, depending on the effectiveness of the handwashing process assumed.

Significance: The model shows key points in the virus transmission process that lead either to further spread or to reduction in the spread of the virus. It has the potential to reduce the morbidity and economic loss currently associated with NoV outbreaks, and to identify key foodservice worker behaviors that affect virus transmission.

P2-54 A Quantitative Risk Assessment Model for *Staphylococcus aureus* in Non-thermal Processed Japanese Foods
DSC

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Introduction: Preventing outbreaks of staphylococcal food-poisoning from Japanese foods requires a holistic approach to Japanese food safety. The typical approach taken is to construct a quantitative risk assessment model (QRAM) in a computer spreadsheet, using probability distributions to model the variability and uncertainty of important risk factors.

Purpose: The purpose of the present work is to conduct a QRAM for *S. aureus* in non-thermal processed Japanese foods and to assess the risk of staphylococcal food-poisoning using Monte Carlo simulation.

Methods: 25 g of raw materials were diluted with 225 ml of peptone water and homogenized in a stomacher. Environmental samples were tested by means of wet swabbing method by using a Swab Rinse Kit. The samples were investigated for the presence of *S. aureus* according to the standard method for enumeration of *S. aureus* in foods. A QRAM for *S. aureus* and Japanese foods was constructed in an Excel spreadsheet and was simulated with @Risk settings of Latin Hypercube sampling and 10,000 iterations, a spreadsheet add-in program.

Results: In sensitivity analysis, 'consumption temperature' came in first with 0.419, followed by 'storage time' with 0.374. In the case of sushi, 'storage time after cooking' contributed significantly to the *S. aureus* level. A scenario analysis indicated that in sushi, the storage time and temperature contributed significantly to a *S. aureus* level above 5 log CFU/g. A concentration of *S. aureus* equal to or exceeding 5 log CFU/g was assumed to be unsafe in the current study. In room temperature sushi, the corresponding values were 3.908 log CFU/g and 2 h. As a result, we suggest that sushi be stored at temperatures of less than 10°C and consumed within 2 h of purchase.

Significance: Simulation results of the present work suggest that QRAM can be a useful tool to assess the risk of pathogen growth in foods and to make decisions to manage food safety for Japanese restaurants located in Seoul.

Poster

P2-55 An Evaluation of Food Safety Practices and Customer Perception of Food Safety Standards within Farmers' Markets in the UK

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Introduction: Farmers' markets are re-emerging as a retail route for food purchasers. The development of these markets offers an opportunity to evaluate food safety practices in an emerging market, which has no easily available access to food safety professionals.

Purpose: The purpose of this research was to evaluate food safety practices by businesses (usually farms) in farmers' markets and to investigate customer perception of food safety standards within this environment.

Methods: The research was conducted through observation practices at twelve farmers' markets and a structured survey questionnaire of stall holders and customers was completed (n = 50).

Results: Social change in the demand for improved traceability of food products and the need for farmers to increase profit margins on primary products has led to an increase in the demand for farmers markets in the UK. The aim of the research was to evaluate food safety practices and perception of food safety standards amongst customers. Twelve farmers' markets were visited to evaluate food safety practices, and 50 attendees from these farmers' markets were interviewed for their perception of food safety standards in the market. A further 50 potential customers who had not visited farmers' markets were interviewed to assess their perception of food safety standards in the markets. Forty percent of stall holders were meat based (lamb/beef), 20% cheese/dairy and 10% craft, with the remainder (30%) miscellaneous food, e.g., bakery, honey, and confectionery. There was a mixture of raw and cooked products on sale. Fifty percent of the meats stall holders had unrefrigerated product on display. Products were on display for an average of 3 h and up to a maximum of 8 h. Thirty percent of farmers not attending cited the lack of non refrigerated units as a reason for their non-attendance, and 90% of these highlighted the spoilage of product, not food safety, as the reason for wanting refrigeration. None of the attendees from a consumer perspective highlighted food safety as a weakness of the market (largest negative response was based on poor marketing of the markets). Of the non attendees group of consumers, 25% cited poor food hygiene practices at the farmers markets as a specific weakness that restricted their attendance.

Significance: The development of farmers' markets as a retail outlet poses a significant risk to consumer safety. This research has identified various contamination risks and has established consumer perceptions of food safety risks associated with this form of retail sales.

P2-56 Microbiological Assessment for Development of GAP Model for Soybean Farms
DSC

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Introduction: The increase in the number of foodborne outbreaks associated with agricultural products has suggested the need to apply Good Agricultural Practices (GAP) in agricultural farms during cultivation and harvest.

Purpose: This study analyzed microbiological hazards in soybean farm environments to develop GAP model for production of safe soybeans.

Methods: Samples of cultivation environments (soil, irrigation water) and plants (soybeans with shells, soybean leaves) were collected from three soy bean farms in August, September, and October of 2008. The collected samples were used to enumerate aerobic plate count (APC), coliform and *Escherichia coli* on plate count agar, desoxycholate lactose agar and eosin methylene blue agar, respectively, and *Escherichia coli* O157, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* were also enumerated on MacConkey sorbitol agar, Baird Parker Agar, mannitol-egg yolk-polymyxin agar and *Listeria* selective agar, respectively. Following the incubation of plates at 30-35°C for 24 to 48 h, biochemical tests were used for further confirmation (three replications, three samples each).

Results: In general, no differences ($P \geq 0.05$) of bacterial populations in samples were found among farms. The levels of APCs (3.1 to 6.9 log CFU/g, ml, or leaf) and coliforms (1.9 to 5.7 log CFU/g, ml, or leaf) were higher ($P < 0.05$) in soybean and soybean leaves than those (soil: APC 6.0 log CFU/g, coliform 3.6 log CFU/g; water: APC 3.5 log CFU/ml, coliform 1.9 log CFU/ml) of cultivation environments. *E. coli* and *L. monocytogenes* were below detection limits in all samples, but 9.1% and 39.4% of the samples contained *E. coli* O157 and *B. cereus* at low level (≤ 1.2 log CFU/g, ml, or leaf), respectively. Proportion of the samples contaminated with *S. aureus* was 9.1% with ≤ 0.6 log CFU/g, ml, or leaf.

Significance: These results suggest that systematic management in soybean farms should be applied to improve food safety of agricultural products, especially for soybeans and leaves. The results from this study should also be useful in developing a GAP model for soybean farms.

P2-57 Microbial Analysis to Establish Good Agricultural Practice in Agricultural Products Processing Center for Perilla Leaves
DSC

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Introduction: Recently, consumption of fresh products has increased beacuse of the health benefits, but foodborne outbreaks related to fresh product consumption have increased.

Purpose: This study identified risk factors that may cause cross-contamination of foodborne pathogens and the need to establish Good Agricultural Practices (GAP) in agricultural products processing centers for perilla leaves.

Methods: All microbial samples were collected before and after the work shift. Samples were prepared from water used for washing with water and ozone, and rinsing. Perilla leaves from each washing step were also collected, and workers (glove, clothing) and surroundings (basket, door knob, packaging table, wrapping bag) were sampled by swabbing.

Levels of aerobic plate counts (APC), coliforms, and *Escherichia coli* were enumerated on plate count, desoxycholate lactose and eosin methylene blue agar, respectively. *Escherichia coli* O157, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* were also enumerated on McConkey sorbitol, Baird-Parker, mannitol-egg yolk-polymyxin and *Listeria* selective agar, respectively, and biochemical tests were used for confirmation.

Results: APCs and coliform bacterial populations in water increased significantly ($P < 0.05$) by less than 2.3 and 1.7 log CFU/ml after work, respectively, while *E. coli* and pathogens (*E. coli* O157, *L. monocytogenes*) were found to be below the detection limit (0 log CFU/ml). After work, bacterial populations in workers and surroundings were not increased significantly ($P \geq 0.05$) as compared with those before work, but *S. aureus* (< 1.7 log CFU/100 cm^2) was detected at high rate (13~50%) of contamination. Although perilla leaves passed through the washing steps, APCs and coliform bacterial populations on the final products were higher ($P \geq 0.05$) than those on unwashed perilla leaves.

Significance: These results suggest that inappropriate washing may spread pathogens. Thus, sanitary conditions of the washing step should be improved, the areas for washing and storage should be separated, and the area where workers are working should also be separated from the storage area, to minimize cross-contamination. Moreover, GAP system should be applied in the facility for microbial safety.

P2-58 Comparison of Transfer Rate for *Listeria monocytogenes* on Workers' Hands and Pork Meat in Pork Processing

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Introduction: In exposure assessment for food processing of whole microbial risk assessment, models of cross-contamination or transfer that can occur in food processing are very important.

Purpose: This study describes cross-contamination events that occur in animal food processing using the example of pork processing.

Methods: We analyzed the number of *Listeria monocytogenes* contamination transferred from pork meat to workers' hands (wearing polyethylene gloves; PG, cotton gloves; CG, and bare hands), cutting boards and knives, and vice versa.

Results: Transfer rate of CG (100.00%) was higher than that of PG (1.51%) and bare hands (2.51%). In particular, when CG were worn, the transfer rate from the CG to bare hands with CG was 0.08%. Also, the range of transfer rates from the contaminated pork meat to cutting board and knife was 0.35 ~ 3.79%. In contrast, the transfer rates from the workers' hands (with PG/CG and bare hands) to cutting board, knife, and pork meat ranged from 0.0012 to 0.441%. There was a lower transfer rate from workers' hands than from pork meat.

Significance: These findings indicate that the use of PG compared with CG could effectively reduce or prevent cross contamination and provide important information concerning the consecutive transfer of *Listeria monocytogenes* during food processing.

P2-59 Microbiological Evaluation of Representative Vegetable Dishes in Korea

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Introduction: The Korea Center for Disease Control and Prevention reported that Koreans ate 1,283 g of food daily in 2007, broken down into 80.7% vegetables, fruits and grains and 19.7% meat, fish or dairy products. However, many outbreaks of foodborne disease have been attributed to consumption of vegetable dishes.

Purpose: The purpose of this study was to obtain information on a microbiological quality of vegetable dishes in order to support the development of quantitative risk models, as well as providing data for development of the computer-assisted HACCP program.

Methods: Three representative samples such as pickled radish strip, seasoned spinach and bean sprout purchased in a university foodservice facility, were analyzed for aerobic plate counts, coliforms, and *Staphylococcus aureus* counts. A sensitive and reproducible TMC-1000 system modified real-time PCR was applied for identification of *S. aureus*.

Results: The aerobic plate count ranged between 5.54 log CFU/g and 7.86 log CFU/g, with the highest count recorded for pickled radish strip. The highest proportion of samples fell in the range between 6.5 log CFU/g and 7.0 log CFU/g. Coliform counts were between 2.8 log CFU/g and 6.5 log CFU/g. The highest incidence level was 31.4% for total coliform counts between 3 log CFU/g and 4 log CFU/g. *S. aureus* counts ranged from 2.43 log CFU/g to 4.18 log CFU/g. The largest grouping (21.3%) of vegetable samples fell between 2.5 and 3.5 log CFU/g.

Significance: This study is expected to be available as the reference for the basal data of pathogens, and moreover the results are useful for identifying microorganisms associated with vegetable dishes in Korea.

P2-60 Food Safety Knowledge and Behavior of Food Handlers and Assessment of Food Service Premises at UAE DSC University Boy Hostels

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Introduction: Foodborne illness imposes economic costs on society and reduces the quality of life of those falling ill. There is strong statistical evidence that the incidence of food poisoning caused by caterers is greater than that caused by any other food sector. More than 1,000 male students consume their meals in United Arab Emirates University hostel's restaurants. Theoretically, they are at high risk of getting food poisoning from meals they eat.

Purpose: The purpose of this study was to evaluate the food safety knowledge and practice among food handlers at United Arab Emirates University Hostels' restaurant.

Methods: A written questionnaire was prepared and distributed to the catering unit's operation manager. The food safety knowledge questionnaire was designed to obtain information about food handlers' demographic characteristics, attitude and practices and knowledge of food safety. Part 1 included seven questions related to demographic characteristics and food safety training. Questions asked about food handlers' attitude and practices in the second part.

Results: The results of the study showed that around 61.5% of food handlers had secondary school education, and 11.5% of the population had a bachelor's degree. Approximately 38.5% of participants had been employed between 3 and 10 years. A positive attitude was reported by the great majority of food handlers, as they answered that they always wear a cap, masks and gloves and that they take off protective clothing before using the toilet and before touching cooked foods. However, some variation was observed in how they washed their hands. The majority (88.5%) of food handlers answered that they always washed their hands with water and soap, while few (11.5%) answered that they washed their hands with water only. The food safety knowledge of food handlers was very good. This may be due to the fact that many of the participants (92.3%) had training in food hygiene and safety.

Significance: The findings of this study demonstrate that the food handlers working at restaurants at the boy's hostel of UAE university have good knowledge of food hygiene and food safety. However, more training can be imparted to them on temperature control. In addition, proper facilities to hold hot food hot and cold food cold should be added to the premises. Further, we recommend that they follow a HACCP system to ensure food safety.

P2-61 Analysis of Microbial Populations Present on Checkstand Conveyor Belts in Grocery Stores

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Introduction: Checkstand conveyor belts in grocery stores could be a potential contamination source for foodborne pathogens. Not much microbial information on these conveyor belts has been documented.

Purpose: The objective of this study was to assess the microbial populations present on checkstand conveyor belt surface in grocery stores.

Methods: A total of 100 samples were collected from 42 grocery stores randomly selected in MI. Two or 3 conveyor belts from each store were sampled, using pre-moistened 1-ply composite tissue (CT) for an area of 10 × 10 cm. For microbial analysis, 40 ml of sterile neutralizing buffer was added in each Whirl-Pak® bag and homogenized by stomaching. The aliquots were appropriately diluted in 9 ml of buffer solution. One ml suitable diluents was pipetted onto 3M Petrifilm™ plates for total aerobic bacteria count (TAC), yeast and mold (YM), *E. coli*/coliforms (EC), and *Staphylococcus* (Staph) and counted at 2, 5, 2, and 2 days after incubation, respectively. The USDA FSIS protocols were followed to test for *Listeria* and *Salmonella*. For analysis of methicillin-resistant *S. aureus* (MRSA), the samples were enriched in D-manitol salt broth and streaked onto Baird-Parker agar plates with Cefoxitin and incubated for 48 h. The microbial populations were presented as log CFU/cm^2.

Results: TAC, YM, and *S. aureus* were found from all the checkstand belts with populations of 5.8, 3.2, and 1.9 log CFU/cm^2, respectively, indicating poor hygiene on the belt surface across the grocery stores tested. There were 8 samples (8%) found positive for coliforms. However, no *E. coli*, *Listeria* and *Salmonella*, and MRSA were found from all the samples.

Significance: Although no foodborne pathogens were found, high microbial populations were present on checkstand conveyor belts. This finding reinforces the need for sanitation practices in grocery stores.

P2-62 Frequency of *Salmonella* spp. in Five Commercial Brands of Chicken Eggs Using a Combined Method of Enrichment and Nested-PCR

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Introduction: Egg or egg-based foods, raw or undercooked, have been identified as vehicles of *Salmonella* outbreaks. The low numbers of *Salmonella* in eggs makes difficult its detection in order to conduct frequency studies. Nested-PCR technique shows more sensibility and specificity than bacteriological methods. A pre-enrichment method followed by enrichment and Nested-PCR represents a good alternative for the investigation of *Salmonella* in eggs.

Purpose: To investigate the frequency of *Salmonella* spp. in chicken eggs using an enrichment procedure followed by Nested-PCR.

Methods: Two thousand chicken eggs representing 5 commercial brands (400 eggs per brand) were purchased from three grocery stores from June to September (summer season). Ten eggs of each brand were combined in order to obtain 200 pooled samples. Both shells and yolks of each pooled sample were individually analyzed. The detection method consisted of pre-enrichment in trypticase soy broth supplemented with 0.6% yeast extract (TSBYE), followed by selective enrichment in tetrathionate broth (TT) and then by Nested-PCR.

Results: From the 100 egg shell pooled samples analyzed, 16 tested positive for *Salmonella* spp., while only 3 of the 100 egg yolk pooled samples tested positive for the pathogen. The isolation frequency of *Salmonella* spp. in egg shells was higher for brand A (6%), followed by brand B (4%), brand E (4%) and brand C (2%). With respect to egg yolk samples, only brand B, C and E were positive for *Salmonella* spp. (1% positive samples respectively).

Significance: The isolation frequency of *Salmonella* spp. in egg were high with respect 0.25% founded in a study performed on year 2005 in Mexico, where bacteriological method was used. The combination of pre-enrichment in TSBYE, followed by enrichment in TT and Nested-PCR is useful for *Salmonella* spp. detection in foods where the expected number of cells is low, or the viable but not cultivable state could be present.

P2-63 Efficiency of Commonly Available Sanitizers and Household Compounds against *Listeria monocytogenes* Biofilms on Food Contact Surfaces with/without Exposure to Nutrients

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Introduction: Listeria monocytogenes cells may adhere to various food contact surfaces, including those in households, and, if these surfaces are not properly cleaned, biofilms can form that can be resistant to sanitizers.

Purpose: We examined the persistence of *L. monocytogenes* on high density polyethylene (HDPE) and polypropylene (PP) food contact surfaces in the presence/non presence of nutrients, and compared the effectiveness of six common sanitizers/household compounds in reducing pathogen levels on surfaces.

Methods: HDPE (rough and smooth surface) and PP (smooth) coupons (2×5 cm) were inoculated (6.0 to 7.0 log CFU/cm^2) with a 5-strain composite of *L. monocytogenes* in ham homogenate. HDPE coupons were stored at 25°C and PP coupons at 25°C or 4°C for up to 21 days. In repeated 24-h cycles, 0.3 ml diluted broth was deposited on the inoculated surface of one-half of coupons to simulate nutrient-rich use, then rinsed with 10 ml distilled water 8 h later and stored 16 h (starvation); additional inoculated coupons were stored throughout without added broth. Sanitizer solutions (one each lactic acid-, quaternary ammonium-, acetic acid-, and hydrogen peroxide-based and two sodium hypochlorite-based) were applied to coupons at 0, 0.25, 1, 4, 7, 14 and 21 d storage. Coupons were analyzed for pathogen (PALCAM agar) and total microbial (Tryptic Soy Agar with 0.6% yeast extract) counts.

Results: Multi-species biofilms, containing 5.0 to 6.0 log CFU/cm^2 *L. monocytogenes*, developed and survived up to 21 days on all surfaces at 25°C, with survival greater on HDPE than PP surfaces and on coupons with repeated nutrient exposure. All products were effective against *L. monocytogenes* on coupons stored at 4°C or without nutrients. At 25°C, all products were effective on coupons sanitized within 24 h, but increasingly ineffective thereafter (2 to 4 log CFU/cm^2 survival on HDPE surfaces sanitized on day 21).

Significance: The results indicated that repeated exposure of food contact surfaces to nutrients, as during use with no cleaning or sanitation, increases the resistance of *L. monocytogenes* biofilms to sanitizers. To reduce such risk, consumers may consider treating surfaces with products such as vinegar when commercial sanitizers are not available.

P2-64 Evaluating Consumer Preparation of Burgers through Video Analysis
DSC

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Introduction: Consumers may expose themselves to foodborne illness through their food preparation methods. Cross-contamination through the contact of raw, potentially contaminated meat with hands or kitchen surfaces and inappropriate temperature management are of microbiological concern.

Purpose: To evaluate consumer food handling practices through analysis of video footage of consumers as they prepare burgers and a salad.

Methods: Volunteers were filmed in their homes as they prepare burgers from frozen burger patties and a salad from whole head lettuce, tomatoes and celery. Burgers were cooked on an outside grill, a stove, or a double-sided electric grill. Following meal preparation, a questionnaire was administered to assess awareness of safe food handling practices, food safety outbreaks and food irradiation. Video footage was evaluated with a score sheet based on the FDA Food Code. Behaviors assessed in the videos include handwashing method and duration, cross-contamination events and burger cooking practices.

Results: Analysis of over 50 households indicate about 67% of handwashing lasts less than 10 s, with an average duration of 6 s. Cross-contamination occurs frequently, with an average of 25 cross-contamination events per household. While 60% of volunteers own a food thermometer, only one used a thermometer to determine doneness of burgers. Burgers were pronounced done at an internal temperature of less than 160°F by 35% of households. Most volunteers have heard of burger-related foodborne illnesses (85%) and *E. coli* (100%), but only 41% of volunteers consider themselves to be most responsible for the safety of ground beef served to their family.

Significance: Consumers expose themselves to foodborne illness through inadequate handwashing, cross-contamination and insufficient cooking, despite increased awareness of foodborne illness. Thermometer use is not widespread. The need for food safety education among consumers persists, with emphasis on protective practices including handwashing, avoiding cross-contamination, selecting irradiated beef, and using thermometers.

P2-65 Repeatability Study of ATP Hygiene Monitoring Systems in Sixty-six Food and Beverage Manufacturing Sites in the United States

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Introduction: ATP hygiene monitoring systems are used to verify that cleaning of manufacturing equipment and the manufacturing environment has been carried out effectively. A key aspect of these (or any) testing systems is the repeatability of the test — how likely is the test to give the same answer when measuring the same level of ATP is measured. Repeatability has very practical implications in the food testing environment as it has a direct effect on the prevalence of false positive and false negative results.

Purpose: The purpose of the experiments reported here was to compare the repeatability of the ATP testing system currently in use by a food manufacturer. By dosing swabs with a defined amount of ATP, the precision of the testing systems could be directly compared in the food manufacturing plant.

Methods: At each location, 10 Clean-Trace Surface ATP swabs and 10 swabs from the manufacturing plant's ATP system were dosed with 25 microliters of and ATP solution. The swabs were activated and measured as per the manufacturer's directions and the results recorded in a spreadsheet. The Coefficient of Variation (CV) was calculated for each system by dividing the standard deviation of the measurements by the mean.

Results: The results were analyzed on the basis of individual head-to-head comparisons and in aggregate, by averaging the CV's determined in each individual comparison. The Clean-Trace ATP system had a lower CV in every comparison. In aggregate, the systems performed as follows: Biocontrol CV = 45.95% (n = 90), Charm CV = 34.16% (n = 280), Hygiena CV = 35.74% (n = 180), Neogen CV = 94.05% (n = 160), 3M CV = 12.11% (n = 660).

Significance: The repeatability of ATP Testing Systems has direct quality and financial implications for the food processor. Both immediate decisions (re-clean or release for production) and long term decisions in the plant are based on data from ATP testing systems, and these decisions may be faulty if based on faulty data, ultimately resulting in financial loss and increased risk.

P2-66 Removal Effects of Electrolyzed Water against Bacterial Biofilms

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Introduction: Microbial biofilms are more resistant to sanitizers, disinfectants and antimicrobial agents than planktonic cells; even with cleaning and sanitation procedures, bacteria were found on equipment surfaces. Thus, the biofilms in food processing facilities need more effective cleaning and sanitation procedures. Although electrolyzed water (EW) has been proved to have strong antimicrobial activities against most pathogenic bacteria, research on the effects of electrolyzed water on bacterial biofilms is rare.

Purpose: The purpose of this study was to evaluate the effects of strong acidic electrolyzed water (SEW) and weak acidic electrolyzed water (WEW) on *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 and *Vibrio vulnificus* biofilms.

Methods: Each 2 mL of cell culture (10^8 CFU/mL) of *L. monocytogenes* ATCC 15313, *S. aureus* ATCC 12692, *E. coli* O157:H7 ATCC 25922 and *V. vulnificus* ATCC 27562 was separately added to a tube containing 25 mL of fresh broth (tryptic soy broth for *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7, and brain heart infusion broth with 1% NaCl for *V. vulnificus*) into which one stainless steel (SS) chip was immersed. The biofilm was allowed to develop on the SS at 37°C for 8 days. Three methods were used to remove adherent cells from SS by SEW and WEW: swabbing (a pre-moistened swab in PBS for 20 times per side), scraping with a Teflon spatula, followed by swabbing; and ultrasonic washing (sonicated in ultrasonic washer for 3 min and then vortexed for 1 min). The biofilms of four bacterial strains on stainless steel (SS) type 304 surface were treated by 10 ppm to 50 ppm of available chlorine concentration of SEW or WEW for 15 s to 10 min.

Results: Submersion of stainless steel chip attached with bacterial biofilm in SEW (50 ppm of available chlorine concentration) within 5 min reduced the cell counts from 5.9 to 2.1 log CFU/cm^2 for *V. vulnificus*, from 6.7 to 1.0 log CFU/cm^2 for *E. coli* O157:H7, from 5.4 to 3.2 log CFU/cm^2 for *L. monocytogenes*, and from 4.9 to 3.2 log CFU/cm^2 for *S. aureus*. When treatment time was increased to 10 min, 50 ppm of SEW could completely eliminate *L. monocytogenes* and *S. aureus* biofilm. Almost identical results were obtained from WEW treatments.

Significance: SEW and WEW could effectively kill viable cells in biofilms and eliminate the bacterial secretion (extracellular polymeric substances) of bacteria on a surface and obviously reduced the available chlorine concentration and treatment time compared with a chlorine solution. Thus, SEW or WEW is a potential disinfectant agent in food processing facilities.

P2-67 Control of *Listeria monocytogenes* on Contact and Non-contact Surfaces by Electrostatic Spraying of Quaternary DSC

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Introduction: The attachment of bacteria on food processing equipment and in the environment after sanitation can cause potential cross-contamination, which can lead to surface destruction, food spoilage, and possible food safety concerns.

Purpose: The purpose of this research was to determine if electrostatic spraying of quaternary ammonium compounds would provide a more efficient means of sanitizing food contact and environmental surfaces to reduce bacterial attachment and prevent biofilm formation.

Methods: Ceramic tile, FRP (plastic wall board), polypropylene conveyor belt-mesh top (24% open mesh) and stainless steel conveyor – single loop (80% open mesh) were inoculated with a *Listeria monocytogenes* (LM) cocktail with a final concentration of 10^6 CFU/ ml and then subjected to either an air pressure spray or an electrostatic spray treatment using 200 ppm of ala-quaternary ammonium.

Results: There were significant ($P < 0.05$) reductions in the amount of LM that remained on the surfaces after being treated with both the electrostatic spray and the air-pressure spray, but no significant differences between the two treatments ($P < 0.05$). To determine which treatment could prevent biofilm formation, the ceramic tile, FRP, stainless steel coupons (306 food grade), and polyethylene (plastic cutting board) were treated with either an air pressure spray or an electrostatic spray treatment using 200 ppm of ala-quaternary ammonium. Biofilms were allowed to form onto the surfaces for 24 h. The biofilms were measured by crystal method analysis and scanning electron microscopy (SEM). The crystal method analysis indicated that electrostatic spray significantly ($P < 0.05$) reduced the biofilm formation on all the surfaces, and the SEM confirmed the absorbency reading.

Poster

Significance: This study determined that the electrostatic spray could potentially be implemented into a sanitation program. This study also demonstrated that electrostatic spray was superior as a pretreatment application.

P2-68 Reduction of *Salmonella* on Five Different Conveyor Belts during Continuous Spray Sanitizing

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Introduction: Conveyor belts have been frequently targeted as a contamination source in meat and poultry processing facilities with much ongoing work in the development of new belting materials and designs that can be more easily cleaned and sanitized.

Purpose: This study assessed the side-by-side performance of two newly designed and three currently used conveyor belts against *Salmonella* during continuous spray-sanitizing.

Methods: The following five conveyor belts were used: Modular acetyl (M-AC) (interlocking, new design), Modular HDPE (M-HDPE) (interlocking, previous design), ThermoDrive polyurethane 1 (TD-PU-1) (smooth, new design), ThermoDrive polyurethane 2 (TD-PU-2) (smooth, previous design) and a 2-ply reinforced fabric polyurethane (2-ply-PU) (continuous) belt. All belts were inoculated in tandem using a 2-track 12 ft long conveyor system by simultaneous passage through an inoculation tray holding 10% turkey slurry with a 3-strain *Salmonella* cocktail (10^7 CFU/ml) for 4 min followed by tray removal and air drying for 15 min. Both belts were then continuously sprayed (30 L/h) with the organic acid sanitizer Vortexx (2340 ppm). At pre-determined intervals, two modular belt segments (15 × 6 cm) and one laced fabric belt segment (15 × 15 cm) were removed and sonicated in 150 ml of neutralizing buffer for 10 min, whereas the two ThermoDrive belt segments (15 × 15 cm) were swabbed using 1-ply composite tissues and homogenized in 30 ml of neutralizing buffer. Healthy and injured *Salmonella* were quantified on XLT4 overlayed with a non-selective medium using either direct plating or membrane filtration. Meat residue on the conveyor belts was also quantified by weighing the belts before and after inoculation and air-drying for 15 min.

Results: After a 25 min exposure to Vortexx, greatest *Salmonella* reductions were seen using the newly designed TD-PU 1 (5.13 log, $P < 0.05$) followed by the M-AC (3.26 log) and 2-ply-PU (4.0 log). Non-significant differences in reduction ($P > 0.05$) were seen between the M-AC and M-HDPE or between the TD-PU-1 and TD-PU-2 belts. Meat residue attachment to the belts was significantly ($P < 0.05$) higher for the M-AC and M-HDPE belts compared to the TD-PU-1, TD-PU-2 and 2-ply-PU belt. No significant difference in meat residue attachment was seen between the TD-PU-1 and TD-PU-2 belts, whereas the M-AC belt yielded significant less ($P < 0.05$) attachment than the M-HDPE belt.

Significance: ThermoDrive belts with smooth surfaces offer greater ease in cleaning and sanitizing compared to traditional modular and fabric belts.

P2-69 Decontamination of Red Radish Seeds Artificially Contaminated with *Listeria monocytogenes*

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Introduction: The use of germinated seeds as food originated in Far Eastern countries and has recently spread to the western world, where sprouts are considered fashionable and healthful ingredients. However, outbreaks of foodborne illness due to the consumption of raw sprouts have been reported. It has been recommended that seeds used to produce sprouts receive one or more treatments during sprout production to assure the safety of raw sprouts.

Purpose: The antibacterial effect of seed decontamination during presoaking before sprouting as an intervention step for eliminating foodborne pathogens on sprouts was evaluated. The effect of seed decontamination on the quality and stability of seed germination rate was also evaluated.

Methods: Red radish seeds were inoculated (at a level of 3 to 4 log CFU/g) with *Listeria monocytogenes* ATCC 19111 and decontaminated with calcium hypochlorite, chlorinated water, acidic electrolyzed water, low-alkaline electrolyzed water, and ozonated water. The control seeds were immersed in distilled water. The germination rate was measured on each treatment for 48 h.

Results: APC and counts of *L. monocytogenes* decreased by 3 logs after immersion in each of five treatments. Treatments with calcium hypochlorite and electrolyzed water were more effective than treatments with chlorinated water and ozonated water. The germination rate ranged from 82 to 96%. After sprouting, APC and *L. monocytogenes* counts on seeds treated with acidic electrolyzed water and low-alkaline electrolyzed water were lower (1 to 2 logs) than the control.

Significance: The results suggest that effective on-farm controls, such as seed decontamination, should be considered in order to reduce the risk of transfer of pathogens associated with consumption of raw sprouts.

P2-70 Protective Effect of *Salicornia herbacea L.* on Acrolein-induced Cytotoxicity Using Human Carcinoma Cells in Vitro

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Introduction: A halophyte, *Salicornia herbacea L.* is an annual succulent shrub that grows on salt marshes and has been consumed as seasonal vegetable as well as in folk medicine for treatment of intestinal ailments. Acrolein is a highly-reactive, α, β-unsaturated aldehyde pollutant to which humans are exposed through the consumption of foods fried in high-temperature cooking oil. It has been implicated in the development of atherosclerosis and various lung diseases, including chronic obstructive pulmonary disease.

Purpose: The objective of this study is to investigate the protective effect of *Salicornia herbacea L.* on acrolein-induced cytotoxicity, using human carcinoma cells in vitro.

Poster

Methods: The fresh aerial part of green *Salicornia herbacea L.* was extracted with methanol and the extraction was sequentially fractionated using hexane, chloroform, ethyl acetate and butanol. 25μM acrolein was added to the A549 human lung carcinoma and HepG2 human hepatoma cells for one hour, followed by washing with PBS. Cells were incubated in new media with fractionation extracts of *Salicornia herbacea L.* at various concentrations (0 to 100 μg/mL), and cell viability was estimated by MTT assay. Inhibitory rates against acrolein-induced cytotoxicity were calculated.

Results: In vitro, exposure of A549 human lung carcinoma and HepG2 human hepatoma cells to acrolein resulted in time- and concentration-dependent cell death. Among the fraction extracts tested in the present study, the fraction of *Salicornia herbacea L.* extracted with butanol resulted in complete recovery of the acrolein-induced cytotoxicity in both A549 and HepG2 cells at 100 μg/mL concentration.

Significance: The present work suggests that butanol fractions of *Salicornia herbacea L.* might be used to reduce the cytotoxicity due to the acrolein produced during food processing. Further study is needed to evaluate various functional effects of *Salicornia herbacea L.*, against various cells.

P2-71 Establishment and Validation of an Analytical Method for Detection of Zearalenone in Medical Herbs by HPLC DSC

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Introduction: Fusarium species have been known to be causative agents of tricothecene mycotoxins. *F. graminearum*, *F. culmorum* and *F. crookwellense* produce a life threatening estrogenic toxin called zearalenone in foods, and feeds as well as in medical herbs.

Purpose: For the quantitative detection of zearalenone in medical herbs, this study established and validated an analytical method using high performance liquid chromatography fluorescence detection (HPLC-FLD) after immunoaffinity column clean up.

Methods: Two clean up procedures based on Solid Phase Extraction (SPE) and immunoaffinity columns after extraction with acetonitrile/methanol water at different concentrations and different dilution buffers (distilled water, phosphate buffer saline with / without Tween 20 and phosphate buffer pH. 8.0) were compared. Samples were extracted with NaCl, 80% MeOH, diluted with 2% Tween 20 in phosphate buffer saline (PBST), and cleaned up through SPE cartridges and immunoaffinity columns prior to HPLC analysis. For validation, medical herb samples (Gwal-loo-in, Doo-choong, Bok-boon-ja, Sook-ji hwang, Shin-E) were artificially contaminated with zearalenone at 100, 200 and 500 ng/g, to evaluate precision and accuracy. Specificity, range, limits of detection (LOD) and quantification (LOQ) were determined on chromatograms of zearalenone artificially contaminated samples. Linearity (R2) of the standard curve (5, 10, 20, 50, 100, 200, 500, 1000 ng/g) was also evaluated.

Results: Immunoaffinity columns after extraction with 80% MeOH and dilution with 2% Tween 20 in PBS, provided precise, accurate and consistent results in comparison to SPE cartridges. Linearity (R2) of the standard curve was 0.9995, and precision (relative standard deviation: 0.39 to 4.40) and accuracy (recovery: 76.59 to 105.95 %) were in the acceptable range of 70 to 120%. LOD and LOQ were 5 and 8 ng/g respectively, with an analytical detectable range from 5 to 2000 ng/g.

Significance: Since there is insufficient information on the natural occurrence of zearalenone in medical herbs, the established validated method may be a useful basis for zearalenone surveillance studies.

P2-72 Study on Control of *Aspergillus* spp. and Aflatoxin B1 in Feed by Gamma Irradiation DSC

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Introduction: Aspergillus spp. are widespread in Korea and all over the world on feeds and foods. They have been known to produce aflatoxins, which are mutagenic and carcinogenic to humans.

Purpose: The objective of this study was to determine the optimum gamma-irradiation dose for reduction and control of *Aspergillus* spp. and aflatoxin B1 in feeds.

Methods: The suspension of fungal spore (10^7 spores/ml) was prepared in 0.85% NaCl containing 0.1% Tween 80 by a cheese cloth method and exposed to 0, 0.5, 1, 2 and 3 kGy of gamma-irradiation, followed by spreading on potato dextrose agar (PDA). The optimum gamma-irradiation dose to reduce fungal growth was estimated by checking fungal colony and size on PDA and AFB1 producibility in culture media. AFB1 level in culture media were tested by direct competitive-enzyme linked immunosorbent assay (DC-ELISA). To confirm growth inhibition of fungi on feeds by gamma-irradiation, the feeds artificially inoculated with *Aspergillus* spp. (10^7 spore/5 g) were prepared and exposed, with radiation ranging from 0 to 3 kGy. Additionally, aqueous solutions of AFB1 (0~5 ng/ml) were irradiated up to 5 kGy and then the AFB1 level was determined by DC-ELISA.

Results: Gamma-irradiation has effectively inhibited fungal growth. In case of fungal spores in aqueous solution, fungal growth appeared on PDA when it was exposed to ≤1 kGy of gamma-irradiation. However, no fungal growth was observed with irradiation ≥ 2 kGy. With ≥ 2 kGy of gamma-irradiation fungal spores artificially inoculated in feed were completely inactivated. The effectiveness of gamma-irradiation to aqueous solution and feed was the same. The fungal spores irradiated with ≥ 2 kGy produced no AFB1 (< 0.6 ng/ml), whereas ≥1 0 ng/ml of AFB1 was detected in fungal spores cultivated without gamma irradiation. AFB1 level at 5 ng/ml was reduced approximately by 85% using a minimal irradiation dose (2 kGy). The results indicate that Aspergillus spp. and AFB1 on feed could be controlled by 2 kGy of gamma-irradiation.

Significance: Gamma-irradiation has proved to be an effective and safe treatment that could be used as a method to reduce fungal growth and AFB1 in feeds.

Poster

P2-73 DSC Comparing the Effectiveness of Vortexx™ and Hydrogen Peroxide to Inactivate *Bacillus* Species Spores Embedded in Food Matrices on Various Food Contact Surfaces

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Introduction: Bacillus anthracis was long considered a potential biological warfare agent before its intentional release through the US postal system in 2001. This incident brought public awareness and concern to various industries, including the food industry. The use of chemical sanitizer treatments is presently the most commonly used method to inactivate spores.

Purpose: In this study, spores of *Bacilllus cereus* ATCC 21218 (Bc), *Bacillus thuringiensis* ATCC 33680 (Bt) and *Bacillus anthracis* Sterne 34F2 (Ba) were mixed with peanut butter or baked sucrose matrix and soiled onto coupons made of actual materials used in food processing plants (stainless steel 316, glazed tile, teflon, polypropylene and Buna-N).

Methods: The coupons were washed with a detergent and treated with either Vortexx™ or hydrogen peroxide, using a clean-out-of-place (COP) method, and the overall inactivation of Bc spores was assessed.

Results: An overall reduction of > 5 log was observed when a 15% liquid or vaporized H_2O_2 was applied to Bc spores embedded in peanut butter on stainless steel coupons and > 6 log reduction was obtained for stainless steel coupons soiled with baked sucrose. Similar results were observed for spores from each *Bacillus* strain on each food contact surfaces, except polypropylene and Buna-N. Because of its non-ovenproof characteristics, these materials were alternatively soiled with baked sucrose and spores and similarly treated, using 5% liquid or vaporized Vortexx™, which resulted in an overall reduction of 4 log spores.

Significance: Therefore, based on our current observations, the use of H_2O_2 was more effective than Vortexx™ for the inactivation of Bc spores on various food contact surfaces soiled with food matrices.

P2-74 Barriers to Contamination by Food Workers

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Introduction: The Committee on Control of Foodborne Illness (CCFI) was tasked with reviewing the role of the food worker in foodborne illnesses. To date, CCFI has collected and published data on outbreaks, contributory factors, survival of the agents and their transfer to and from hands, and has shown that workers have many opportunities to contaminate food, resulting in illnesses.

Purpose: The last component is to examine barriers which can reduce the likelihood of pathogens contaminating a food during its processing or preparation.

Methods: The literature was reviewed to obtain specific studies on barriers, which was sometimes conflicting in results.

Results: Barriers have focused on respiratory contaminants such as sneeze guards and avoiding direct hand contact with the product. Unfortunately, aerosols that include viable microorganisms are frequent although the levels are low, and norovirus outbreaks have been linked to aerosols from vomiting, which sneezeguards cannot prevent. Clothing, utensils, deli papers, and packaging are all designed to limit direct hand to food contact but are not consistently used. Food operations, when displaying food, should protect food from potential sources of contamination including: ensuring that the packaging remains intact during storage; protecting unpacked food and newly prepared food from dust, dirt, pests, touch and aerosols; and maintaining food at their correct temperatures. Although utensils have hygienic value during food production and preparation, for ease of working, hands need to be in regular contact with food much of the time, and glove use has been advocated to prevent direct transfer of pathogens. However, although gloves have been demonstrated to reduce transfer of pathogens to surfaces in experiments, outbreaks have been associated with glove use by workers. In all cases, the gloves were not worn consistently, not worn to cover skin lesions, or were not worn during particular food preparation and handling operations like adding garnishes. Other limitations to glove use include a false sense of security, pinhole leaks because of improper manufacture or puncture by rings or long or artificial nails. Another issue is that occlusion by gloves increases discomfort and growth of microorganisms in contact with the moist skin, with leakage of microorganisms onto food if the gloves are not effectively used. Removal of gloves at this stage, without proper handwashing, presents a significant hazard because wet hands facilitate the transfer of contamination.

Significance: Barriers are important, but they need supplemental actions, including effective hand washing, to minimize contamination of Ready-to-Eat food by humans.

P2-75 Acid Resistance of Biofilm and Planktonic *Lactobacilli*

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Introduction: Lactic acid bacteria (LAB) are noteworthy spoilage bacteria of foods and pose a constant concern in the food industry. They have the potential to form biofilms on the production line or in raw materials and cause contamination or deterioration of many types of food.

Purpose: The purpose of this study was to investigate the change in resistance of biofilm and planktonic LAB to acid stress, which strongly inhibits bacterial growth and is important in food preservation.

Methods: The responses of biofilm and planktonic cells of *Lactobacillus* strains that originated from food materials or human feces were investigated using survival tests. The differences between these cells before and after the test were compared by observation under scanning electron microscopy and confocal laser scanning microscopy. The gene expressions of these cells were compared by microarray analysis.

Results: The biofilm bacterial cells of all the *Lactobacillus* strains tested showed greater resistance than the planktonic bacterial cells in organic acids. The gene expression patterns were different between the biofilm and planktonic bacterial cells, particularly in relation to the cell surface or extracellular proteins and carbon source metabolism.

Significance: The change in stress resistance and gene expression in the biofilm bacterial cells demonstrated the importance of controlling biofilms of LAB in the food industry.

P2-76 Phosphine Fumigation for *Salmonella* Enteritidis Control in Black Pepper (*Piper nigrum*) in Grains

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Introduction: Phosphine fumigation, a method widely applied for insect control in stored grains, can be an alternative for controlling *Salmonella* Enteritidis (SE.) in black pepper in grains thus decreasing rejection of lots by importing countries due to the presence of this pathogen.

Purpose: To determine the effect of different phosphine treatments on the control of SE in black pepper in grains at low and high water activities.

Methods: Samples of irradiated black pepper in grains with a_w of 0.67, 0.92 and 0.97 were inoculated with SE and submitted to phosphine concentrations of 0.0, 3.0 and 6.0 g/m^3 for 24, 48 and 72 h at 35°C. Post fumigation effects were also verified. Phosphine was measured by gas chromatography and SE countings in the pepper samples by dilution plate technique. Water activity was also monitored.

Results: Phosphine fumigation, when applied to the product at high water activities, at concentrations of 6 g/m^3 for 72 h, decreased around 80% of the SE population. No SE was observed in fumigated grains 72 h after drying, while in the controls 60% of SE was still recovered.

Significance: The study indicates that phosphine fumigation can be applied to moistened black pepper grains before drying as an additional treatment for the control of SE. After the fumigation, producers should rigidly follow good agricultural practices, mainly during grain drying, in order to avoid product re-contamination.

P2-77 Adiafood Solution for Pathogen Detection in Less Than 24 Hours

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Introduction: Adiafood PCR kits (formerly Warnex) were designed for easy use in industrial laboratories testing for food pathogens (*Salmonella, Listeria, E. coli* O157:H7, *Campylobacter*). A new instrument, the Stratagene Mx3005P driven by a new version of the software, Sentinel2, is now available for Adiafood solutions while keeping the AOAC RI validation (simplex kits). Also a new generation of kits (called duplex kits) with Internal Control is now available for *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes* all 3 of these methods have a very short TTR (Time To Result) of 21 h.

Purpose: As *L. monocytogenes* is a slow grower compared to others, most of the work was done on this pathogen.

Methods: First, a new medium dedicated to PCR method called Listerboost was developed to reach a sufficient amount of cells after only 18 h enrichment (less latency growth and better growth speed). This medium avoids any problem of amplification inhibition or fluorescence cross reading. Second, the extraction protocol was modified accepting a bigger enrichment take to increase sensitivity. Two centrifugation steps were included using 96 format tubes to concentrate cells before lysis.

Results: Evaluations were made on several types of naturally contaminated products coming from meat, milk and seafood. Out of 595 samples, results show a 91% concordance between Adiafood *L. monocytogenes* and the reference method (ISO11290). And discordant results are mainly due to positive results with PCR that are negative with the reference method; most samples positive for PCR are confirmed by petri dish analysis (ALOA, plating 100 µL) due to a higher sensitivity. Finally, testing very different types of products demonstrated that inhibition levels of the Adiafood *L. monocytogenes* kit were exceptionally low (3%) even with difficult foods such as chocolate desserts or cheese.

Significance: Compared to other methods currently available on the market, the Adiafood *L. monocytogenes* kit with only 21H TTR is probably the fastest method for food *L. monocytogenes* detection. The new Adiafood duplex kits for *L. monocytogenes* and *Salmonella* will be validated this year according to the ISO16140 standard and the AOAC RI.

P2-78 Withdrawn

P2-79 Removal of Potentially Allergenic Residues from Stainless Steel Surfaces

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Introduction: Foodborne allergens continue to pose food safety hazards to at-risk consumers despite regulatory scrutiny and processor strategies for allergen control and removal. Many sanitation standard operating procedures (SSOPs) rely on the implementation of validated allergen removal protocols to ensure the safety of their products produced in manufacturing plant settings, and these SSOPs are embedded within HACCP and other safety plans. However, there is little scientific literature that provides specific information about tested validation protocols for the removal of potentially allergenic residues.

Purpose: The purpose of this research was to validate the removal of egg, peanut and milk residues from stainless steel using a range of cleaning protocols.

Methods: Potentially allergenic food products (peanut butter, liquid egg and milk) were mixed into an inoculum applied in a controlled manner to polished stainless steel. The food contact surface was subjected to several cleaning protocols that encompassed a water rinse, and the application of low, medium and high detergent levels (corresponding to 100 to 150,

450 to 550, and 900 to 1,000 ppm active alkalinity, respectively) and two different cleaning solutions (71°C and 90°C) average infeed temperatures with at least 10 s of combined spray and cascade solution dwell time prior to water rinsing. Visual scales measuring degree of residue of wet/dry surfaces were developed to assess typical cleaning inspection protocols. Allergen residues were tested with commercial test kits (Alert Allergen Test Kits, Neogen Corporation, Lansing, MI) in conjunction with the development of a standard curve.

Results: In all wash combinations, the level of each potentially allergenic residue was reduced from approximately 1 g protein/100 cm^2 to at most 1 µg/100 cm^2, thus validating wash procedures for this specific system. A level of 1 µg/100 cm^2 or less was defined (in this study) as the complete removal of significant allergen residue. Wet/dry visual residue assessment scales did not always agree with allergen test kit results.

Significance: Wash protocols can be demonstrated to provide for the complete removal of allergen residues in a practical sense. A complication in this type of research is the reliance on test kits for the determination of specific allergenic residues, which seem to provide the most robust means of testing cleaning protocols, as well as the continued need for commercially relevant regulatory definitions of significant levels of allergens.

P2-80 Building ISO 22000 Compliant Food Safety Systems

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Introduction: The ISO 22000 standard defines a food safety management system. This combines food safety management programs such as HACCP with other functions such as quality, supply chain management, and ingredient and product genealogy. Since most HACCP programs are predominately paper based, the question is how to integrate the food safety function into modern information management systems.

Purpose: The purpose of this presentation is to identify the components required for such an integrated food safety/quality management system, identify the conversion process and provide a road map for implementing an ISO 22000 compliant system.

Methods: The method is to review the ISO 22000 standard and to identify existing information management systems which can be integrated to provide a compliant food safety management system.

Results: Existing quality information systems can be extended to support HACCP operations and comply with standards-based and regulatory requirements. Such a food safety and quality system can be integrated with supply chain management and genealogy systems to meet the requirements for the food safety management system defined in ISO 22000. In addition to managing the operations, there is greater data integrity and visibility which when combined with analytics and reporting increases food safety performance and responsiveness to food safety incidents.

Significance: The road map allows processors to identify the areas where they need to enhance their safety and process management system. By implementing this process it is feasible for food processors to integrate existing process management, genealogy, and tracking systems to build and maintain an ISO 22000 compliant food safety management system.

P2-81 Denaturation / Renaturation Kinetics of Staphylococcal Enterotoxin in an Acidulated Food Matrix

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Introduction: Staphylococcal food poisoning is caused by the ingestion of toxins produced by some strains of *Staphylococcus aureus* and other strains of staphylococci. In highly acidic environments, denaturation of the toxin can occur, thus destroying the serological activity of the toxin with retention of toxicological activity.

Purpose: The detection of the staphylococcal enterotoxin is dependent on the use of classical in vitro serological methods. Denaturated toxin cannot be detected serologically. The general purpose is to renature the toxin so that serological detection can occur.

Methods: Five-500 mL batches of tomato juice (TJ) were prepared and labeled TJ + Toxin (TX), pH 4.27 (original pH of product); TJ + TX, pH 3.0; TJ + TX, pH 3.5; TJ + TX, pH 7-8 (positive control) and TJ + no TX (negative control). The batches of TJ, other than the negative control, contained 20 ng each of toxin serotypes A, B, and D per mL of juice. The denaturation and renaturation behaviors of the toxins were monitored by ELISA approximately every two weeks to track serological destruction of toxin in these acidulated environments. Restoration of serological activity was achieved by exposure of the toxin molecule (TJ, pH 4.27; TJ, pH 3.0 and TJ, pH 3.5) to 6.0 to 8.0 M urea + 0.354 g zinc acetate per mL of food product.

Results: Toxins in an acidulated matrix resulted in partially or completely denatured states and were renatured with urea and zinc acetate thus making serological identification of the toxin possible.

Significance: Environmental stress, such as heat or acidity, as with food processing, alters the antigenic integrity of staphylococcal enterotoxin. Exposure of denatured toxins to renaturants, such as urea and zinc acetate, restores the antigenicity to the toxin so that the toxin can be serologically identified. Human consumption of denatured toxin is a viable approach for the intentional contamination of acidulated foods.

P2-82 Rapid Testing of Non-dairy and Mixed Dairy Beverages Using the 3M Microbial Luminescence System (MLS, formerly Cogent)

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Introduction: ATP testing systems have been used for several years in rapid testing for commercial sterility in Ultra High Temperature (UHT) and Extended Shelf Life (ESL) dairy products. With the increasing prevalence of smoothies, teas, and coffees being blended with dairy products, there are new applications for ATP testing systems in these more complex matrices.

Purpose: The purpose of these experiments is to evaluate the usefulness of existing and modified dairy sterility testing reagents for non-dairy and dairy-derived products.

Methods: A variety of dairy, non-dairy, and dairy-derived beverages were tested, using the MLS system and the MLS reagents with modified compositions. The beverages were inoculated with low levels of common microbes and spoilage organisms, incubated for 24 to 48 h; the results of the MLS system were compared with results of plating methods to determine sterility.

Results: The modified MLS reagents were able to successfully reduce the background ATP of a wide variety of beverage matrices, while the standard dairy reagent kit was unable to reduce the ATP background in many matrices. The modified MLS reagents were similarly successful in detecting microbial contamination in the tested products following incubation.

Significance: The ability to rapidly detect microbial contamination in otherwise sterile products has important financial and quality implications. A kit that is able to perform reliably in a wide variety of matrices would be convenient for manufacturers that produce a wider variety of products than simple dairy beverages.

P2-83 Quantitative Risk Assessment for *Salmonella* in Raw, Frozen Chicken Products
DSC

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Introduction: Recent salmonellosis outbreaks have been associated with consumption of frozen products containing raw poultry. Epidemiological investigations report that consumers perceive these products as being precooked, and improper cooking (often using a microwave oven) was identified as a common practice leading to infection.

Purpose: The objective of this study was to provide a quantitative estimation of the risk of salmonellosis associated with consumption of raw, frozen chicken products, utilizing published and newly collected data.

Methods: A quantitative risk assessment using @Risk was developed. Data on *Salmonella* prevalence and concentration, consumer cooking practices and microwave use, and *Salmonella* dose-response was taken from the published literature. Thermal inactivation data for microwave cooking was collected in our laboratory. Frozen chicken entrees inoculated with *Salmonella* were cooked in a microwave oven, according to the label's instructions, at 500, 1000 and 1300 Watts. Temperatures were monitored by use of fiberoptic thermometry.

Results: Frozen chicken entrees cooked at 1000 or 1300 Watts reached mean final temperatures of 93 to 99°C and 99.5 to 101°C respectively; *Salmonella* was not recovered from any of these samples. Entrees cooked at 500 Watts reached mean final temperatures of 42 to 63.5°C, and *Salmonella* was recovered in every case. A recent outbreak in Minnesota linked to frozen chicken entrees (4 reported cases) was simulated, using our risk model. Actual cases predicted by the model ranged from 12 to 36 for 100 simulations of this outbreak. Using an alternative simulation approach, raw, frozen chicken products were estimated to be responsible for 6.75% of the total number of *Salmonella* infections per year.

Significance: This study demonstrated experimentally that the use of a low wattage microwave oven for cooking frozen products containing raw poultry is an unsafe practice. The results of the risk assessment developed may provide useful quantitative data relevant to risk management initiatives that are ultimately aimed at controlling the risk of salmonellosis from raw, frozen chicken products.

P2-84 Thermal and Chemical Inactivation of Ricin in Orange Juice
DSC

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Introduction: The potential use of ricin as a bioterror weapon in the food supply is a major concern for homeland security. Thermal treatments can reduce the toxicity of ricin in water solutions, but its stability in foods subjected to heat processing is largely unknown.

Purpose: The objective of this project was the identification of heat and chemical treatments capable of inactivating ricin toxin in orange juice so that it can be disposed of safely in the event of an attack.

Methods: Diluted ricin was mixed with orange juice for inactivation studies. The ricin concentration in samples was determined using an ELISA immunoassay. Thermal stability was determined in capillary tubes using an oil bath at high temperatures typical of pasteurization. For chemical inactivation, sodium hypochlorite (NaOCl), sodium hydroxide (NaOH) and peracetic acid (PA) were added alone or in combination to samples before thermal treatment. The Arrhenius model was used to evaluate temperature dependence.

Results: Heat inactivation of ricin followed first-order kinetics (i.e., $\log_e$ Conc vs. time). The half-life ($t_{1/2}$) of ricin at 72, 80, 85 and 90°C was 60.5, 4.86, 1.72, and 0.23 min, respectively. The Z value was 7.4°C indicating high temperature sensitivity. After 1.25 min at 90°C, the concentration of residual ricin was approximately 3% ($6t_{1/2}$). No residual ricin in juice was detected at 80°C for 60 s when treated with 0.2% NaOCl and 0.15 N NaOH, and after 90 s with 0.1% PA. At room temperature, no ricin was detected within 5 s in juice after treatment with 2% NaOCl, 0.2 N NaOH or 0.64% PA. These results indicated synergism between NaOCl and NaOH and considerable efficacy with treatment with PA alone.

Significance: This study delivered the first series of time/temperature/biocide concentration conditions that would serve as the basis for recommendations for treating juice subjected to intentional adulteration with ricin so that it can be disposed of safely.

P2-85 Rapid Discrimination of Sanitizer-stressed Microorganisms by Fourier Transform Infrared Spectroscopy (FTIR)

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Introduction: Microorganisms go through different physiological or structural changes when exposed to stressful conditions such as acid, heat, cold or chemicals. Chemical sanitizers are often used post-cleaning to reduce microbial

contaminants on inanimate surfaces to levels that are considered safe. Even though use of chemical sanitizers in food manufacturing plants is to minimize growth of pathogens or spoilage, stressed microorganisms may persist in these environments due to cellular changes. Fourier Transform Infrared Spectroscopy (FTIR) has been used to characterize different microorganisms based on unique spectral features of cellular constituents such as lipopolysaccharides, proteins, phospholipids and nucleic acids.

Purpose: This study was designed to evaluate the use of FTIR to discriminate selected microorganisms that were sanitizer-stressed and non-sanitizer stressed.

Methods: Lactobacillus malefermentans ATCC 11305, *Lactobacillus plantarum* ATCC 8014, *Cronobacter sakazakii* ATCC 12868, *Salmonella* Typhimurium ATCC 13311, and *Salmonella* Enteritidis ATCC 13076 were used in the study. Each of the 24-h cultures were exposed to each treatment (500 ppm octanoic acid, 400 ppm quaternary ammonium compound, 400 ppm active chlorine and sterile deionized water as the control) for 5 min. These cultures were neutralized, centrifuged, and washed with sterile deionized water. The final pellets were resuspended in sterile deionized water and dried to be read using the FTIR.

Results: Characteristic absorbance peaks were observed at wavelengths between 1600 and 800 cm^{-1}. The peaks were of different shapes and intensities for both bacterial strain and treatment conditions. The majority of the differences between the controls and the stressed bacteria were observed at the amide protein, ester and alkyl peaks. This suggests a change in the protein expression levels in response to stress, and may indicate an increase in the lipid bilayer of the cell.

Significance: These results suggest that FTIR can be a rapid tool to discriminate between sanitizer-stressed and non-sanitizer stressed microorganisms under the tested conditions.

P2-86 DSC Determination of Walnut Content in Foods and Environmental Swabs by Enzyme Immunoassay

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Introduction: Walnut is considered to be one of the most common of the tree nut allergies and with an apparent increase in prevalence and the potentially life threatening severity of reaction, walnut allergy represents a significant health concern. It is estimated that approximately 1% of the European and US populations suffer from an allergy to nuts (peanuts and tree nuts, including walnut). With the inclusion of walnut in the food allergen labelling laws of various countries, there is a need for reliable detection and control of this allergen.

Purpose: The BIOKITS Walnut Assay Kit was developed for use in the detection and quantification of walnut at very low concentrations in cooked and uncooked foods, as well as on environmental swabs. The assay utilises polyclonal antibodies to walnut protein in a non-competitive, sandwich ELISA by means of five discreet standards: 2.4, 6, 12, 60 and 120 ppm whole walnut.

Methods: In order to evaluate the BIOKITS Walnut Assay Kit, the following validation parameters were investigated and determined:

Results: Sensitivity (Limit of Detection) 0.25 ppm whole walnut; Inter-assay variability – 7.96% Coefficient of Variation (CV); Intra-assay variability – 6.31% CV; Extraction variability – 14.22 %CV; Cross reactivity – Pecan (2.3%); Quinoa (0.0012%); Pistachio (0.0009%); Hazelnut (0.0005%); Buckwheat (0.00024%); Robustness – 7 variations in the extraction and enzyme immunoassay were tested with no significant effect on the assay functionality; Sample matrices and interference – 100% of foods containing walnut were detected by the assay. 95.5% of foods not containing walnut were correctly identified. Several commodities were evaluated for walnut spike recovery with a determined range of recovery of 56% to 117%; Environmental swab testing – environmental walnut protein surface contamination was detected at least as low as 100 ng / 25 cm^2.

Significance: This study demonstrates that the BIOKITS Walnut Assay Kit is a reliable, robust, specific and highly sensitive assay for the detection and quantification of walnut. Its suitability for use in the detection of undeclared walnut within cooked and uncooked foods, as well as environmental swabs, means that the BIOKITS Walnut Assay Kit can be employed as a key part of allergen control programs.

P2-87 DSC Optimizing Sample Preparation Methods, Monitoring and Risk Assessment of Ethyl Carbamate in Traditional Korean Fermented Foods Using Gas Chromatography/Mass Spectrometry (GC/MS)

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Introduction: Ethyl Carbamate (EC) that occurs during fermenation is classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC). It was reported that EC was in traditional Korean fermented foods.

Purpose: The aim of this work is to optimize sample preparation methods according to food matrix and accomplish monitoring and risk assessment of EC, using GC/MS.

Methods: Sample preparation for EC analysis using an internal standard (butyl carbamate) and GC/MS was optimized according to food matrix. 128 kinds of fermented foods were purchased in Dept. stores and traditional markets. EC in the foods was analyzed based on the optimized sample preparation methods.

Results: A celite-alumina column was used for the analysis of EC in kimchi, fermented paste and salted seafood as the optimized method. For fermented paste and salted seafood, distilled water was used as solvent in extraction process. The level of EC in fermented paste ranged from not detected to 240.2 ppb. EC was undetected or quantified in kimchi and

salted seafood. EDI (Estimated Daily Intake), CDI (Chronic Daily Intake), ECR (Excess Cancer Risk) and MOE (Margin of Exposure) of EC in this study were 2.0 ng/kg-b.w./day, 1.5 ng/kg-b.w./day, 7.5 × 10-8 and 153,061, respectively.

Significance: This study will be useful to use for regular monitoring and to set a limit on EC content of foods, and to establish an analytical method for EC in traditional Korean fermented foods.

P2-88 Comparison of Allergen-specific (ELISA) and Non-specific (Visual Inspection, ATP Swabs, Total Protein Swabs) Methods for the Detection of Soy-based Food Residues

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Introduction: Effective cleaning of shared processing equipment is a major strategy for reducing the risk of allergen cross-contact. Therefore, there is interest by food manufacturers to develop effective cleaning methods and analytical methods for verifying their effectiveness. Although cleaning of shared equipment has been identified as one of the critical points for allergen control, there is little published information on methods that can be used to verify the efficacy of cleaning procedures for removal of soy food residues.

Purpose: The objective of this study was to compare allergen-specific (ELISA) and non-specific (ATP, total protein, visual inspection) methods for detecting the presence of soy food residues in solution and on food-contact surfaces.

Methods: Soy flour, soy milk and soy-based infant formula powder were diluted with water to obtain solutions/slurries containing 0-50,000 µg soy product/mL. Solutions of the soy products were analyzed with two different soy ELISA kits, sensitive and conventional ATP swabs, and a total protein swab to determine the limit of detection of each method. All solutions were analyzed at least in triplicate. One mL of each of the soy-based food solutions was pipetted onto the surface of stainless steel, urethane, and Teflon plates. The plates were placed in an oven (80°C; 1 h) to obtain dried food residues. The plates were visually examined for presence of residues and then swabbed for ELISA, ATP and total protein testing. All trials were done at least three times. Results of analytical tests were considered positive when all trials resulted in detection of the soy-containing foods.

Results: The detection limits for the analytical tests (ELISA, ATP, total protein) for soy flour in solution were 100 ppm. Total protein swabs detected 100 ppm soy milk and soy infant formula solutions while the other methods were not able to detect these foods until concentrations were > 500 ppm. The total protein swab had the lowest detection limits for soy flour and soy milk dried on the three food-contact surfaces compared to the other methods. For the soy infant formula, the detection limits for visual inspection and the total protein swab (1000 µg) were lower than those for the other methods studied here.

Significance: This study shows that allergen-specific and non-specific methods are valuable tools for detecting the presence of soy food residues on food-contact surfaces and in solution, but limitations exist and care must be taken when choosing a method. For the types of soy foods studied here, visual inspection and total protein swabs were the most sensitive methods for detecting the presence of soy residues.

P2-89 Detection and Identification of Psychrotrophic *Clostridium* spp. from Spoiled Vacuum-packaged Fresh Beef

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Introduction: Commercial episodes of "blown" vacuum packed meat tend to occur when meat is temperature abused; however, there have been cases of "blown pack" spoilage that have occurred in vacuum packaged meat stored at refrigeration temperatures (-1.5 to 2 degrees Celsius). These packages exhibit gross distention of the package and production of off odors with 4 to 6 weeks of storage. Psychrotrophic *Clostridium* species have been implicated in "blown-pack" spoilage.

Purpose: The objective of this study was to use PCR to detect psychrotrophic *Clostridium* spp. that caused "blown pack" spoilage of vacuum packaged fresh beef and to isolate and identify novel species.

Methods: Purge was obtained from commercial "blown-pack" spoiled meat. One sample of purge was subjected to heat treatment, the second to ethanol treatment, and the third was enriched in peptone-yeast-glucose-starch broth. DNA was obtained from the purge samples and enrichment broth. PCR amplification was performed, using three primers that were designed based on published research and three primers designed using IDT Primer Quest and 16S rDNA sequence obtained from GenBank. The purge was used for enumeration of anaerobic organisms on Reinforced Clostridial Agar with 5% sheep blood (RCA). One strict anaerobe was isolated from the purge and sequencing of the 16S rDNA was completed.

Results: Using species specific primers, at least two different *Clostridium* spp. known to cause "blown-pack" spoilage were identified in all samples (heat and ethanol treated, and enriched). Sequence of the 16S rDNA of the isolated organism was compared against that of type strains. The sequence obtained was 1.2 kb pairs. Based on PCR with species specific primers, the anaerobe was identified as *Clostridium* spp. but the 16S rDNA did not match that of any *Clostridium* spp. implicated in "blown-pack" spoilage, including the two species detected using species specific PCR. Application of these organisms in meat systems confirmed the role of the *Clostridium* spp. in "blown-pack" of vacuum packaged meat.

Significance: Spoilage of fresh meat results in significant economic losses for the meat industry. Understanding of the microbial ecology of these organisms, including identification, allows technologies and methods to be developed to prevent these organisms from spoiling meat.

P2-90 Immuno Assay-based Test for the Detection and Quantitation of Soy Protein Contamination of Food Commodities

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Introduction: Allergy to soy proteins is a food allergy that accounts for about 3% of allergies in the American population.

Purpose: Develop an immunoassay-based test that can be used in the food industry to monitor soy protein contaminations in incoming ingredients or in the final product.

Methods: A 30-min Soy Protein Allergen Test for quantitative analysis of soy protein contamination in food products and incoming raw ingredients was developed. The test is a Sandwich Enzyme-Linked Immunosorbent assay (S-ELISA). Polyclonal and monoclonal antibodies against specific soy protein markers were used as the capture and detector antibodies. For quantitative analyses, a standard curve of soy protein isolate (SPI) ranging from 0 to 100 PPM (0 to 4 µg ml) was used. Samples were extracted by shaking 5 g of ground samples with 125 ml of PBS in a hot water bath. Extracts were filtered and filtrates were used directly for ELISA analyses. Extracts are added to antibody-coated wells in which soy proteins bind to the capture antibody during a 10-min incubation period. Any unbound protein is washed away and anti-soy horseradish peroxidase-labeled antibody (detector antibody) is added. The detector antibody binds to the soy protein during a 10-min incubation period. Unbound enzyme-labeled antibody is washed away and a one step substrate is added. Color develops as a result of the presence of bound-labeled antibody during a final 10-min incubation period. Absorbance readings of samples are compared with those of the standards and the concentrations in parts per million (PPM) are calculated.

Results: Mean recovery of soy protein isolate (SPI) from various spiked samples was found to be 87%. Limit of detection was found to be less than 10 PPM (0.4 µg/ml) determined as soy protein isolate (SPI). The test is capable of detecting residue contamination of all sorts of soy products such as soy flour, soy protein isolate, soy protein concentrate, soy textured proteins and most soy processed products. No cross-reactivity was observed with any other plant or animal proteins such as tree nuts, grains, legumes, pork, beef or chicken meats.

Significance: The test can detect microgram quantities of soy contamination in different commodities such as milk, juice, salad dressing, meat products, ice cream and bakery products.

P2-91 A Sensitive, Rapid ELISA Test for the Detection and Quantitation of T-2 and HT-2 Toxins in Grain Commodities

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Introduction: T-2 toxin and HT-2 toxin are potent toxins belonging to the group trichothecenes type A mycotoxins that are usually produced by fungi of the *Fusarium* genus, which are commonly found in various cereal crops (wheat, corn, barley, oats, and rye) and processed grains (malt, beer and bread). T-2-and HT-2 toxins often occur together in infected cereals. They elicit a severe inflammatory reaction in humans and animals and have teratogenic effects.

Purpose: To develop an easy to use, accurate analytical method to detect and quantitate the two toxins in grain commodities.

Methods: A polyclonal antibody-based Enzyme Linked Immunosorbent Assay (ELISA) for the detection and quantification of T-2 and HT-2 toxins in agriculture commodities was developed. The assay is a direct competitive ELISA in a micro-well format.

Results: The detection limit of the assay is less than 1 ng/ml (less than 10 ppb) of individual T-2 and HT-2 or a mix of both mycotoxins. The test can detect T-2 or HT-2 at 100% of either or a mixture of them in corn, wheat, barley , rye and oats. Concentration of T-2 or HT-2 required for 50% binding inhibition is 6.8 ng/ml (68 ppb). The antibody used is very specific for T-2 and HT-2. The antibody has no cross reactivity with other trichothecenes such as T2-tetraol deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, and, fusarenon-x, zearalenone or nivalenol. Samples were extracted by shaking 5 g ground sample with 25 ml of 70% methanol-water (1:5) for 3 min. Extracts were diluted 1:1 with water and then used in the ELISA test. The mean recovery of T-2, HT-2 or a mixture of both in corn, wheat, barley and oats determined by this method was 87%.

Significance: The assay can be used to quantify T-2/HT-2 in samples within 10 min.

P2-92 Comparative Study of the Soleris™ Yeast and Mold Test System and Direct Plating for Semi-quantitative Determination of Yeast and Mold in Foods

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Introduction: The Soleris Yeast and Mold test system is an automated, growth-based method with an optical endpoint. A portion of test sample homogenate is added to the Soleris vial containing a selective growth medium. Products of yeast and mold metabolism cause a color change in an indicator chemical, and this change is detected by the instrument over time. Threshold test sensitivity is determined by the volume and dilution of sample homogenate added to the Soleris test vial. The test may be applied at a single threshold to yield a positive or negative result, or two or more dilutions may be tested to generate a semi-quantitative result. Results are available in 72 hours or less.

Purpose: The purpose of this study was to compare performance of the Soleris method to that of the FDA/BAM direct plating method for determination of yeast and mold levels in 10 food products. Inclusivity and exclusivity characteristics of the method were also assessed in pure culture testing.

Methods: Foods tested included hard salami, apple pie, tomato juice, nonfat dry milk, dry pet food, salad dressing, saw palmetto powder, soy flour, strawberry puree and dry cake mix. Soy flour and strawberry puree were naturally contaminated. Other foods were inoculated with yeast and/or mold (single strains or in combination) at target levels of 0, 20, 200, 2,000 and 10,000 CFU/g. Five replicate samples at each level were tested in parallel by the reference direct plating method and the Soleris method at four different test thresholds (10, 100, 1,000 and 5,000 CFU/g). Method agreement was calculated and results were compared using a Chi-square significance test. Inclusivity testing was conducted with 50 strains of yeast and mold (14 genera, 33 species) in pure culture, and all produced positive results. Exclusivity testing was performed with 30 strains of bacteria (17 genera, 29 species) in pure culture, and all produced negative results even when added to the Soleris vial at concentrations as high as 100,000 CFU/vial.

Results: Agreement between the methods in individual trials ranged from 87 to 98% (89% overall). Chi-square analysis of results of individual trials showed, with few exceptions, that results of the Soleris and direct plating methods were not significantly different.

Significance: Results showed that the Soleris method is an accurate alternative to conventional plating procedures for the semi-quantitative determination of yeast and mold in a variety of foods. The Soleris method offers considerable labor savings and provides results in 3 days or less, instead of the 5 days required by standard direct plating methods.

P2-93 Isotachophoretic Method for the Concentration and Purification of Proteins and Nucleic Acids from Food Matrices

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Introduction: It is envisioned that next generation biosensors will be capable of generating near real time results by use of multiple sensing modalities to yield orthogonal confirmation for the presence of pathogens in difficult matrices. For these systems, sample preparation will remain a critical challenge, requiring simultaneous co-purification of nucleic acids, proteins, lipids, and small molecules in a single, universal approach.

Purpose: The purpose of this study was to evaluate the ability of an isotachophoretic approach for universal sample preparation by simultaneously concentrating and purifying proteins and nucleic acids from spiked food matrices for subsequent detection by immunoassays and real-time polymerase chain reaction assays (RT-PCR).

Methods: A variety of food matrices and selective growth media were spiked with purified *Bacillus atrophaeus* DNA and ovalbumin and tested, using PCR and immunoassay respectively, to determine the effect of the matrix on the quality of the assay result. DNA and protein was added to food matrices or growth media previously determined to significantly alter the quality of the assay result and then purified and concentrated, using a gel-based isotachophoresis approach. The method utilized a "sea level" agarose gel format in which the buffers in the anode and cathode chambers of the gel box touched the gel, but were not allowed to mix. Application of a voltage resulted in the separation of sample components with purified nucleic acids and proteins located at the interface between a set of fast and slow moving electrolytes. Purified DNA and protein were removed from the gel matrix and the level of purification was evaluated based on characterization of the analytes by RT-PCR or immunoassays, depending on the target being measured.

Results: For nine of the 11 matrices tested in this study, isotachophoresis purified target DNA to a point that the PCR result was indistinguishable from amplification of the target when no matrix was present. For the remaining 2 matrices, pepper and cocoa, a significant improvement in amplification efficiency after isotachophoresis was observed, but the amplification efficiency after purification was still poorer than amplification when no matrix was present. None of the matrices significantly affected the immunoassay result.

Significance: This study provided a proof of concept, showing that an isotachophoretic approach to sample preparation could provide a solution for the simultaneous purification and concentration of both nucleic acids and proteins from food matrices.

P2-94 An Independent Laboratory Evaluation of a Real-time PCR Combination *Salmonella* spp. *Escherichia* coli O157:H7 Method after a Common 8-hour Enrichment Compared to the USDA/FSIS Reference Methods for the Detection of *Salmonella* spp. and *E. coli* O157:H7 in Raw Ground Beef

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Introduction: The *Salmonella* spp./*E. coli* O157:H7 PCR method is intended to simultaneously reduce the enrichment time for *Salmonella* spp. from 20 h to 8 h and detect *Salmonella* spp. and *E. coli* O157:H7 from the same sample. This is accomplished using a single non-selective enrichment and DNA extraction protocol.

Purpose: The purpose of this study was to compare the performance of the iQ-Check Salmonella spp. *E. coli* O157:H7 method to the USDA/FSIS (MLG 4.04 and MLG 5.04) reference methods for the detection of *Salmonella* spp. and *E. coli* O157:H7 in raw ground beef.

Methods: In two separate trials, samples were simultaneously inoculated with *Salmonella* spp. and *E. coli* O157:H7. The test method samples were enriched for 8 h in buffered peptone water (BPW) and then the PCR assay was performed. The PCR results were compared to results of the 24 h enrichments for each reference method.

Results: Statistical analysis of Chi Square according to Mantel-Haenszel showed no significant difference in the performance of the test method after an 8-h incubation compared to the 72-h and 46-h USDA/FSIS reference methods for the detection of *Salmonella* spp. and *E. coli* O157:H7. In the two trials, the test method identified 24/40 *E. coli* O157:H7 test samples, which were subsequently confirmed as positive, compared to results with the USDA reference method, which confirmed 19/40 samples. The test method identified 21/40 *Salmonella* spp. test samples, but only 7/40 of these

were confirmed as positive by the USDA/FSIS reference method. Isolation of *Salmonella* spp. on selective agar plates was improved by transferring smaller amounts of BPW enrichment to TT and RVS broths (100 µl and 20 µl, respectively) and resulted in a total of 15/40 confirmed positive samples.

Significance: A real-time PCR method which can detect both *Salmonella* spp./*E. coli* O157:H7 from a single enrichment and with a shortened incubation is a valuable tool for a food safety testing laboratory.

P2-95 Microbial Contamination of Date Rutab Collected from the Markets of Al-Hofuf City in the Kingdom of Saudi Arabia

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Introduction: Rutab (Arabic name) is the first stage of maturity on which date fruits can be consumed as food. It is a fresh product with 35% to 40% water and 45% to 48% sugars (dry basis). Because of its high moisture content, rutab is highly susceptible to microbial growth and spoilage, especially when poor hygienic practices during handling lead to heavy microbial contamination. Microbiological studies on dates are limited, especially in the main countries of production.

Purpose: To investigate the microbial contamination of rutab from different date cultivars grown in the Gulf Region in the Kingdom of Saudi Arabia.

Methods: Forty rutab samples were purchased from different retail outlets at weekly intervals, 10 samples at each sampling time, and microbial analysis was carried out on the same day. The fruits (4 pieces) were washed with 50 ml sterile peptone water in sterile stomacher bags, and the aliquots plated in duplicate as 10-fold dilutions. Aerobic mesophilic bacteria were enumerated on PCA, coliforms on VRBA, yeasts and molds on PDA, *Staphylococcus aureus* on medium No. 110, and *A. flavus/parasiticus* on AFPA. *E. coli* O157 was identified using the *E. coli* O157 Latex Test and *S. aureus* using the Staphylase Test, both from Oxoid.

Results: All of the 40 samples tested were found contaminated with aerobic mesophilic bacteria at loads of the order of 10^2 to 10^5 CFU/cm^2 with some significant differences among varieties that can be attributed to differences in the weather conditions during rutab season. Almost all samples were contaminated with molds and yeasts at loads of the order of 10^2 to 10^3 CFU/cm^2. Potentially pathogenic *S. aureus* was detected in all samples, and *A. flavus/parasiticus* in 13 samples, while coliforms were detected in 25 samples.

Significance: Some rutab samples were contaminated with high loads of aerobic mesophilic bacteria; hence a short shelf life is expected. The fruits were also contaminated with different pathogenic microorganisms, posing potential public health hazards.

P2-96 Evaluation of a Rapid Molecular Subtyping Method for Predicting *Salmonella* Serotypes

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Introduction: Serotyping is a classical phenotype-based subtyping method commonly applied to *Salmonella* isolates; there are over 2,500 *Salmonella* serotypes according to the standard Kauffman-White scheme. Traditional serotyping methods may take several days to determine the complete serotype of an isolate. In addition, serotyping does not have the same discriminatory power for differentiation at the strain level as genetic-based subtyping methods such as pulsed field gel electrophoresis or repetitive extragenic palindromic sequence-based PCR (rep-PCR).

Purpose: The objective of this work was to investigate the utility of the DiversiLab System™, a rapid rep-PCR-based subtyping system, as a tool to predict the serotype of *Salmonella* isolates.

Methods: Eighty-two *Salmonella* isolates from the Silliker Laboratories Research Culture Collection were used in this study. These isolates were previously serotyped using standard techniques and represent over thirty serotypes. Isolate DNA was extracted using a bead-beating method and then used as template for rep-PCR using *Salmonella* strain typing kit reagents. Microfluidic LabChip® devices and the DiversiLab System™ were used to separate amplified fragments and analyze sample fingerprints. Samples were compared against the DiversiLab *Salmonella* library using the "Classification Report" and "Top Match" applications.

Results: Sample rep-PCR fingerprints were compared against 313 fingerprints representing 55 serotypes in the reference *Salmonella* library. The software predicted the correct serotype for 39 strains, including *Salmonella* serotypes Agona (3 samples), Enteritidis (7), Hadar (5), Senftenberg (3), and Typhimurium (3). Thirty-four samples did not match any of the serotypes predicted; eleven serotypes were not represented in the library. Certain serotypes were classified as being closely related based on rep-PCR fingerprint patterns. For example, many Montevideo isolates had both Oranienburg and Montevideo predicted among their top serotype matches.

Significance: These data suggest the potential of automated rep-PCR subtyping as a tool to predict *Salmonella* serotypes more rapidly than traditional serotyping. While further development of a reference fingerprint library is necessary to fully utilize the DiversiLab System™ as a rapid means of serotyping, these data also demonstrate that rep-PCR subtyping provides greater strain-level discriminatory power, as several rep-PCR fingerprint patterns exist for a single serotype.

P2-97 Immunomagnetic Separation of *Listeria monocytogenes* Using Nanosized Beads DSC

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Introduction: Listeria monocytogenes continues to be a major foodborne pathogen that causes food poisoning and in some cases death among immuno-supressed people and abortion among pregnant women. Nanometer-sized beads have recently drawn attention in immunomagnetic separation due to the size effects of particles, large surface area/volume

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ratio, and stability against sedimentation in the absence of an applied magnetic field. These characteristics of nano-sized beads boost the binding efficiency by more than 20 to 30% and the available adsorptive areas by 100 to 1000 times.

Purpose: The objective of the present study is to develop a specific, sensitive, and reproducible immunomagnetic separation concept, using magnetic nanoparticles with a diameter of 30 nm, for highly efficient separation of *L. monocytogenes* without any need for filtration or centrifugation steps.

Methods: Magnetic nanobeads (30 nm) were functionalized with rabbit anti-Listeria monocytogenes antibodies via biotin-streptavidin bond and then amalgamated with the target bacterial cells to capture them. A magnetic field was employed to catch the nanobead-*L. monocytogenes* complex and the supernatants were removed. After a washing step, *L. monocytogenes* was separated from a food sample and was ready for detection.

Results: The preliminary results showed that a capture and separation efficiency of 75% can be attained with 30 nm beads for *L. monocytogenes* in PBS solution, and the total immunoreaction time was 60 min. However, beads with larger diameter (150 nm) gave only ~ 40% capture efficiency, indicating that the developed immunomagnetic nanobeads (with diameter 30 nm) based separation method is beneficial over methods using beads with larger size because of their higher capture efficiency, minimal sample preparation, no need for mechanical mixing, and less background noise in detection. Further work will be concentrated on optimization of conditions of the immunomagnetic separation method and detection of the separated *L. monocytogenes* cells using microfluidic chip based biosensor.

Significance: The outcome of this study will tremendously enhance the separation efficiency of foodborne pathogens, which will provide better prepared samples for further testing.

P2-98 Susceptibility to *Enterobacter sakazakii* Changes with Increasing Age in Neonatal Mice

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Introduction: Premature or very-low-birth-weight infants orally fed reconstituted powdered infant formula unintentionally contaminated with *Enterobacter sakazakii* (*E. sakazakii*) may develop infections resulting in severe outcomes such as septicemia, necrotizing enterocolitis, meningitis, or death. Infants who recover from infection may have morbidities such as hydrocephalus, mental retardation, or developmental delays. Although increasing age appears to reduce susceptibility, it is not known at what age infants become less susceptible to *E. sakazakii* infection.

Purpose: Our objectives were to compare the susceptibilities of neonatal mice of different ages to *E. sakazakii* infection and to identify biomarkers of infection.

Methods: Timed-pregnant CD-1 mice were obtained and allowed to give birth naturally. Neonatal mice were orally gavaged at postnatal day (PND) 1.5, 5.5, and 9.5 with a single dose of vehicle or 10^4, 10^8, or 10^{11} colony-forming units (CFU) *E. sakazakii* strain MNW2 per ml reconstituted powdered infant formula. On post-treatment day 7, surviving pups were sacrificed and brains, livers, and ceca were excised and analyzed for the presence of *E. sakazakii* invasion.

Results: E. sakazakii was isolated from brains, livers, and ceca of neonatal mice treated at PND 1.5 and 5.5 but not from those of pups treated at PND 9.5. *E. sakazakii* was more invasive in brains than in livers and ceca, with total isolations of 25.3%, 21.2%, and 19.7%, respectively. Mortality was observed in neonates treated at PND 1.5 at a total of 11.25%, whereas no deaths occurred in neonates treated at the older ages.

Significance: Like human infants, neonatal mice show a time-dependent susceptibility to *E. sakazakii* infection, with resistance increasing with increasing age. Future work will further characterize the infection and provide a model to develop treatments and therapies for *E. sakazakii* infection in human infants.

P2-99 Evaluation of the Spartan DX™ Real-time Portable PCR Analyzer Using TrimGen eQ-PCR™ STEC-*stx*1 and STEC-*stx*2 Detection Kits for Shiga-toxin Genes

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Introduction: The Spartan DX™ is a 4-well, portable, inexpensive real-time PCR platform. An evaluation of TrimGen eQ-PCR™ Shiga toxin detection kits for *stx*-1 and *stx*-2 genes was performed on two Spartan DX™ real-time PCR analyzers, which combines the three conventional steps of PCR (denaturation, annealing, extension) into two.

Purpose: Evaluation of the Spartan DX™ Analyzer and TrimGen Shigatoxin *stx*1 and *stx*2 detection kits was performed to determine their usefulness and reliability for laboratories requiring non-batched results.

Methods: DNA isolation was performed on 30 select strains of shiga toxin-producing and non-producing bacteria using Qiagen® DNeasy Blood and Tissue kits. *Stx*-1 and *stx*-2 genes were then detected simultaneously on both Spartan DX™ instruments using primer and probe sets for each gene, along with positive and negative controls. Also, diluted DNA samples and inoculated ground beef samples (10^1-10^3 CFU/g) were analyzed to assess the instrument's sensitivity.

Results: Results showed that 23 strains were positive for both genes (22 *E. coli* O157:H7, 1 *Y. enterocolitica*), 3 were positive for *stx*-2 only (*Shigella sonnei, Shigella flexneri, Citrobacter freundii*), and 4 were negative for both genes (*Hafnia alvei, E.coli* O129(B):H11, *E. coli* O157:H7 *stx*1-/*stx*2-, generic *E. coli*). Results for the *Yersinia* and *Shigella* strains indicate possible cross-reactivity of kit reagents with these species. Comparison of the Ct values for each instrument revealed no significant differences ($P > 0.05$). The analyzers succeeded in detecting genes in DNA samples diluted by 6 logs, and in isolates recovered from ground beef samples inoculated with decreasing levels of STEC, indicating a high degree of sensitivity.

Significance: Overall, the Spartan DX™ PCR analyzer was user-friendly, easy to learn, and results were obtained rapidly (one hour following DNA isolation and PCR prep). This instrument could potentially improve the diagnostic capabilities of smaller laboratories or help developing countries obtain much needed molecular detection technology.

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P2-100 Isolation and Identification of Gas-producing Yeasts from Maraschino Cherries

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Introduction: Production of maraschino cherries is a time-consuming and complex process that includes long-term brine storage of the picked fruit, followed by pitting, leaching and reintroduction of various preservatives in cherry syrup followed by bottling. Gas production of unknown origin is an occasional quality defect observed in jars of maraschino cherries.

Purpose: This study was designed to isolate and identify specific yeasts responsible for gas production in maraschino cherries.

Methods: Twenty-eight commercial maraschino cherry syrup samples were collected late in the production process and examined for yeasts by enriching in Carbohydrate Purple Broth containing 1% high fructose corn syrup or corn syrup (pH 6.18-6.52), 50% aqueous cherry syrup (pH 3.66-3.81), and 50% cherry syrup diluted 1:1 in Trypticase Soy Broth containing 0.6% yeast extract (Cherry:TSB-YE; pH 6.19-6.66). Yeasts were isolated on Trypticase Soy Agar containing 0.6% Yeast Extract (TSA-YE), deMan-Rogosa-Sharpe Agar (MRS), and Potato Dextrose Agar (PDA) (48 h/28°C). Gas production from selected isolates was confirmed in the previous enrichment media using Durham tubes, with each yeast isolate biochemically identified with API 20 C AUX test strips.

Results: Twelve of the 28 samples yielded gas-producing yeasts, regardless of the media used, with the greatest level of gas production seen in Cherry:TSB-YE. Based on colony morphology and results from the API 20 C AUX test strips, 10 isolates were identified as *Candida krusei/inconspicua* with one isolate each identified as *Candida magnoliae* and *Candida lusitaniae/famata*. *C. krusei/inconspicua* and *C. magnoliae* only grew on PDA and MRS, whereas *C. lusitaniae/famata* grew only on all three plating media.

Significance: This is the first report of yeasts being responsible for gas production in maraschino cherries. Having identified these gas-producing yeasts, an appropriate intervention strategy such as thermal pasteurization can potentially be developed to eliminate this quality defect in maraschino cherries.

P2-101 Parent Attitudes and Self-reported Handling of Powdered Milk Formula: Implications for Microbiological Safety and Education

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Introduction: Methods used to prepare and store reconstituted powdered infant formula (PIF) have important microbiological implications for safety. Recommended procedures in the home may be achieved by parents equipped with adequate/correct knowledge, positive attitudes and motivation to implement desired behaviors that can minimize microbial risks.

Purpose: This study determines attitudes, risk perceptions and self-reported behaviors related to preparation and storage of PIF inside and outside of the home.

Methods: Structured face-to-face interviews of 200 parents were undertaken in hall-tests in England and Wales, using a Computer-Assisted-Personal-Interviewing technique. Quota controls on age groups and socioeconomic-grading were applied, and the sample was found to be representative of parents who feed their infant with PIF at least once a day.

Results: Findings indicated that methods parents use to prepare PIF are variable. Fifty-two percent of parents reported feeding their infant immediately after reconstitution of formula. However, large proportions (> 35%) of parents prepared feeds in advance of use, a practice perceived to be acceptable by 58% of the sample. Other malpractices reported included storage of feeds at room temperature until required (12%) and reheating feeds more than once (3%). Barriers for implementation, specific methods used for cooling and reheating feeds with reported lengths of time will be discussed within the context of microbial safety. The majority (97%) of parents believed they had full responsibility and full control of hygiene and safety when preparing PIF for their infant; smaller proportions of parents (44 to 73%) believed that 'other parents', day-nursery staff and hospital staff had the same level of responsibility (63 to 82%) and control (44 to 73%). Ninety percent of parents believed there was a very low risk of infant illness after feeding reconstituted PIF they had prepared; risk of illness was perceived to be greater if feeds were prepared by 'other parents', day-nursery staff and hospital staff.

Significance: Findings from this study will help the development of targeted information that address the microbial risks of preparation and storage of powdered infant formula.

P2-102 Food Safety Auditing: An Evaluation of Auditor Variability between High and Low Risk Products

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Introduction: Variability in auditor performance continues to undermine the validity of internationally accredited food safety standards. Minimal research has been undertaken in establishing variation between food auditors applying the same standard to different food groups.

Purpose: Previous research has indicated variation in auditor performance. This research focuses on auditor variability in an accredited standard BRC which should not be affected by high/low risk product status.

Methods: Based on 100 audits, ten auditors who undertake audits for high and low risk food products were identified. All had similar career backgrounds and the last ten audits undertaken by each auditor for high and low risk products were analysed to establish trends in non-conformances. A questionnaire was also developed to establish the auditors approach to auditing both high and low risk product types. Clauses analysed were not product specific, e.g., pest control, glass control.

Results: Eighty percent of auditors felt that they raised more non-conformances that were not product related in high-risk (compared with low risk) food groups. Four hundred fifty-six non-conformances were raised within the research sample. The number of non-conformances (not related directly to product type) was 36% higher in high-risk companies than in low-risk companies. The greatest difference in the number of non-conformances raised was in training, which had 68% higher level of non conformances. Non conformances raised in the maintenance section were 22% higher in low risk product processors than in high risk counterparts. 90% of the companies processing high-risk foods felt that auditors raised more non conformances because of their high-risk status.

Significance: Internationally accredited food standards require consistency in delivery to ensure their survival. This research is important in delivering data on variability and recommending corrective actions to minimize this variability.

P2-103 Detection of VOCs in Spoiling Pork Using Field Asymmetric Ion Mobility Spectrometry

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Introduction: The spoilage of meat during storage and transportation, due to the growth of bacteria populations, is a problem with obvious consequences. There would be obvious loss to profits to a meat company due to subsequent recalls of product, not to mention the loss of consumer confidence. In an effort to conserve fuel, refrigeration systems on trucks are turned off, exposing the meat to elevated temperatures at which bacteria start to grow. This problem occurs on a daily basis as 8 million trucks per day transport meat across the US.

Purpose: Preventing spoilage through appropriate temperature control during transport is highly necessary, as is monitoring meat to detect when it is spoiled. Unfortunately, methods for determining the presence of bacteria can take several days to give results. A rapid method of detection of bacteria is therefore needed.

Methods: Field Asymmetric Ion Mobility Spectrometry (FAIMS) was used to monitor the out-gassed volatiles of pork samples incubated at 25°C over a 24-hour period. During this period, adsorbent trap samples were taken for volatile identification. Bacterial APC counts were determined through traditional growth methods in a petri dish in an agar medium.

Results: Using FAIMS technology, a change in spectrum occurred after 4 h, indicating that the bacteria were present and of sufficient count to produce a volatile organic compounds. This was then correlated to the bacterial count at this time of log 2.96. Meat is considered spoiled at log 8.5. Subsequently, volatile organic compounds were identified by thermal desorption gas chromatography-mass spectrometry (TD-GC-MS). The VOCs identified showed steady signal increases over time on the GC-MS plots and FAIMS Spectra as the bacteria counts increased.

Significance: This method of detection is both simple to use (dock personnel) and gives a result in less than a minute. This will allow meat companies to perform quality control measurements for the presence of bacteria in delivered meat (2000 lb combos). This significantly reduces the liabilities of the meat company and provides protection for the consumer.

P2-104 Antimicrobial Potential of Thirty-two Natural Compounds against Common Juice Spoilage Microorganisms (*Saccharomyces cerevisiae*, *Zygosaccharomyces bailli*, *Z. bisporus*)

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Introduction: Yeasts have the ability to cause food and beverage spoilage, leading to millions of pounds worth of economic losses each year. Spores and vegetative cells of most yeasts are easily inactivated by pasteurization; however, consumer demand for natural products has manufacturers striving to produce fresh tasting juices by minimizing the use of heat treatments, which have the potential to alter sensory characteristics and nutritional value.

Purpose: By use of a Minimum Inhibitory Concentration procedure (MIC), 32 chemical compounds (alcohols, essential oils and parabens, extracted from natural sources) were screened for their ability to inhibit the growth of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailli*, and *Z. bisporus* in a 10% apple juice medium (AJM), as a model beverage (brought to12% brix with the addition of sucrose, glucose and fructose) for up to 12 weeks.

Methods: Fifty microliters of each chemical concentration tested was added in triplicate to AbGene micro-titer test tubes, followed by 950 µl of AJM and 4 µl of yeast inoculum, for a final concentration of approximately 2 log CFU/ml. Tubes were incubated at 30°C and inspected for turbidity daily for the first month and weekly for the last two months.

Results: Nine compounds were capable of significantly extending the shelf life of the AJM ($P \leq 0.05$) beyond twelve weeks. Spoilage occurred with the remaining compounds, at all concentrations tested, within a week. There were no significant differences observed between the different types of yeast ($P \geq 0.05$).

Significance: Identification of new antimicrobials for use in the beverage industry could result in reduced product losses and improved product quality for the consumer.

P2-105 Evaluation of Four Membrane Filter Materials for Use with 3M™ Petrifilm™ *E. coli* Coliform Count Plates to Enumerate *Escherichia coli* in Water Samples

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Introduction: The American Public Health Association Standard Method [SM] 9222B for enumeration of *Escherichia coli* in drinking-water requires filtration of 100 mL water through a membrane filter, followed by plating the filter onto a selective and differential growth medium. 3M Petrifilm *E. coli*/Coliform Count [EC] plates have global method approvals for use within the food industry, but no method validations for use in drinking or bottled water.

Poster

Purpose: The goal of this project was to investigate the feasibility of using 3M Petrifilm EC plates coupled with membrane filters to detect and enumerate *E. coli* in drinking water samples.

Methods: Membrane filters from four manufacturers were evaluated as candidates for use with 3M Petrifilm EC plates. A total of 264 corresponding samples of water were inoculated with *E. coli* ATCC 25922. Half of the samples were inoculated at a "low" concentration of 10 to 50 CFU/100 mL and the other half at a "high" concentration of 100 to 150 CFU/100 mL. Samples were filtered through each of the four membranes, plated onto hydrated 3M Petrifilm EC plates, incubated and enumerated both manually and using the 3M Petrifilm Plate Reader. Comparison of the filter recovery was performed, using Student *t*-tests and ANOVA.

Results: Analysis of data following logarithmic transformation showed counts between the four membranes were not statistically different for low count samples ($P > 0.05$). When comparing all 4 filter recoveries at the high range, a significant difference was observed ($P = 1.40E-09$). *T*-tests revealed that the four filters could be grouped into two statistically similar pairs. Review of images captured by the 3M Petrifilm Plate Reader indicate other factors may potentially affect results: membrane appearance (transparent versus opaque); incubation conditions such as height of plate stacks, location of plate within the stack, and plate moisture loss. The impact of these factors is being investigated.

Significance: These preliminary studies indicate that the 3M Petrifilm EC plate coupled with membrane filtration shows promise for consideration as an acceptable alternative quantitative *E. coli* method to SM 9222B.

P2-106 Efficacy of Supercritical Carbon Dioxide for Inactivating *Lactobacillus plantarum* in Apple Cider

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Introduction: Juice makers have traditionally used thermal pasteurization to prevent deterioration by spoilage bacteria such as *Lactobacillus plantarum*; however, this thermal processing causes adverse effects on product quality such as undesirable taste and destruction of heat-sensitive nutrients. For this reason, nonthermal processing has been investigated and developed as an alternative to thermal pasteurization. Of non-thermal technologies, use of supercritical carbon dioxide ($SCCO_2$) is promising in that carbon dioxide (CO_2) is non-toxic, non-reactive, non-flammable, inexpensive, and environmentally safe.

Purpose: The objective of this study was to evaluate the efficacy of $SCCO_2$ for inactivating *L. plantarum* in apple cider, using a newly developed continuous system with a gas-liquid porous metal contactor.

Methods: Pasteurized apple cider without preservatives was used for this study, and apple cider inoculated with *L. plantarum* was processed using a $SCCO_2$ system at a CO_2 concentration range of 0 to 12% (g CO_2/100 g product), outlet temperatures of 34, 38, and 42°C, a system pressure of 7.6 MPa, and a flow rate of 1 L/min.

Results: Higher CO_2 concentrations and temperatures significantly enhanced microbial reduction, resulting in 5.85 log reduction with 12% of CO_2 at 42°C. The degree of microbial injury increased significantly as CO_2 concentration and treatment temperature increased. Morphological changes in $SCCO_2$ processed cells were observed by SEM. $SCCO_2$ treatment and refrigeration (4°C) effectively inhibited the growth of *L. plantarum* during 28-day storage.

Significance: This study showed that supercritical carbon dioxide treatment is effective in eliminating *L. plantarum* and could be applicable for nonthermal pasteurization of apple cider.

P2-107 DNA Microarray for the Characterization and Typing of *Salmonella*: A New Tool for Risk Analysis

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Introduction: DNA microarrays are going to become one of the most powerful alternative methods for the simultaneous characterization and typing of *Salmonella enterica* isolates. The use of microarrays enables the parallel study of large numbers of virulence determinants and their distribution within the strains investigated within less then three days.

Purpose: The purpose of this study was the development of a new DNA microarray harboring a condensed selection of important genetic *Salmonella* markers for the discriminative characterization of *Salmonella* serovars.

Methods: The DNA microarray of the German National *Salmonella* Reference Laboratory comprises approximately two-hundred and eighty 57-63mer oligonucleotide probes that detect genes associated with metabolic pathways, flagellar and somatic antigen-encoding genes, important virulence genes located within or outside the pathogenicity islands, prophage genes and genes htat are part of, or related to, mobile genomic-elements participating in the horizontal transfer of specific antibiotic resistance genes. The probes were printed on glass slides and hybridized with genomic Alexa555-labelled *Salmonella* DNA.

Results: Twenty-three *Salmonella* strains and one *E. coli* strain were selected as controls for each probe. The validity of the results was confirmed by gene specific PCR or phenotypic methods (serotyping, MIC determination for various antimicrobial agents). The microarray results showed an agreement of 97% between the microarray and PCR/phenotypic methods. Only approximately 3% of overall results were classified as uncertain, were not printed, or showed a discordant result. The new developed print- and hybridization control enables the validation whether all spots were printed and if the hybridization itself worked properly.

Significance: The microarray described here provides a tool for rapid study of not only the epidemiology of *Salmonella* strains at the genomic level but also their pathogenicity and resistance potential. It therefore represents a powerful method for microbial food diagnosis and outbreak investigation, as well as risk analyses and assessment.

P2-108 Internal and Independent Laboratory Validation of PCR Assays for Detection of *L. monocytogenes* from Both Food and Stainless Steel Surfaces

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Introduction: Listeria monocytogenes was characterized more than 75 years ago as a causative agent of disease in animals, but only within the past 20 years has it been widely recognized as an important cause of human foodborne illness. Sensitive methods with faster time to result are needed so that interventions strategies may be implemented more rapidly.

Purpose: Internal and external studies were conducted to evaluate a new PCR assay for the rapid detection of *L. monocytogenes* using a single proprietary enrichment for next-day results.

Methods: For various spiked foods and stainless steel surfaces, one set of twenty spiked replicates and five unspiked controls per matrix were enriched for 18 to 20 hours in 24E broth, then tested with the 24E *L. monocytogenes* assay, using the PCR-based BAX® system. A second set of replicates was tested according to an appropriate reference method. Additionally, 50 *L. monocytogenes* and 50 non-*L. monocytogenes* were tested for inclusivity and exclusivity.

Results: The internal studies showed that the *L. monocytogenes* PCR method, across all five foods/surfaces and organisms, detected 79/120 samples with next- day results compared to 67/120 for the reference method (~5 days). Of the 120 samples, 82 were confirmed for *L. monocytogenes*. The external studies on stainless steel showed that the PCR method detected 14/20 versus 17/20 for the reference method. Of the 14 PCR positives, 14 were confirmed as *L. monocytogenes*. For hot dogs. the PCR method detected 10/20 versus 14/20 for the reference method. All unspiked samples were negative by PCR and culture. One-hundred percent inclusivity and exclusivity was observed. The aggregate internal data indicates that the *L. monocytogenes* PCR method was statistically equivalent ($X^2 = 2.50$) to the reference method.

Significance: The PCR assay performance is equivalent to reference culture methods, but with faster time to results, it can enable intervention strategies to be implemented more rapidly.

P2-109 Development of Multiplex PCR Analysis for Detection of Major Peanut (ARA H 1), Hazelnut (COR A 1) and Almond (PRU DU 2.02) Allergens in Food Products

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Introduction: People suffering from food allergies are dependent on accurate food labelling to prevent the allergic reaction. The detection of the main peanut (*Arachis hypogaea*) allergen Ara h 1, hazelnut (*Corylus avellana*) allergen Cor a 1 and almond (*Prunnus dulcis*) allergen Pru du 2.02 represents the tool of regular food labelling authentication.

Purpose: The purpose of this study was to develop a multiplex PCR method for the detection of three major food allergens (peanut, hazelnut, and almond). Declared and nondeclared food products were investigated for the presence of these allergen residues.

Methods: Three sets of primers for the amplification of the partial genomic sequence of the Ara h 1 (180 bp), Cor a 1 (397 bp) and Pru du 2.02 (249 bp) genes were designed and applied to the investigation of 72 commercial food samples with or without peanut, hazelnut or almond declaration. The specificity of primer pairs was tested. The universal plant primers for the plant matrixes confirmation in food (123 bp) were used.

Results: Thirty samples of food products with hazelnuts, thirteen with peanuts, eight with almonds, 18 without declaration and three with common declaration as nuts were analyzed using the developed PCR method. In non-declared samples no hazelnuts, peanuts or almonds were detected. The detection limit of the method was 0.01%, for hazelnut and almonds, and 1% for peanuts.

Significance: The developed multiplex PCR method is specific enough to detect peanut, hazelnut and almond major allergens simultaneously in food products. Financially supported by grants MZe 00027 16201 and QG 60090/2005.

P2-110 Prevalence of *Bacillus cereus* in Fried Rice Dishes and Its Exposure Assessment from Chinese-style Restaurants in Korea

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Introduction: To conduct a risk profile for *Bacillus cereus* in fried rice dishes, data on the prevalence of *B. cereus* as well as an exposure assessment are needed. Fried rice dishes are one of the most popular food items in Korea; however, there are no data on the exposure pathway and contamination level of *B. cereus* in those dishes.

Purpose: This study aimed to produce an exposure assessment for fried rice dishes and to evaluate the microbiological quality of these foods from Chinese-style restaurants.

Methods: The exposure pathway for fried rice was assessed in terms of time, temperature and serving size by phases from preparation to consumer consumption point, through on-site observation at 8 Chinese-style restaurants in Gyeonggi province. The microbiological quality of 32 samples of fried rice delivered from restaurants was evaluated for the levels of *B. cereus*, ACC, and Coliforms. Chromogenic *Bacillus cereus* agar was used for the presumptive test of *B. cereus*, and real-time PCR was performed with TMC-1000 system for the confirmation test.

Results: One serving size of fried rice dishes was 352.2 g. In 3 restaurants that reheat pre-cooked rice dishes, mean temperature of the dishes after the reheating phase was 68.5°C, and the final cooking time was 1 min 14 s. Meanwhile, mean temperature of fried rice dishes in 5 cook-to-order type restaurants was 81.4°C, with 1 min 49 s of cooking time. Average meal assembly time was measured as 1 min 20 s. Average delivery time from restaurant to place of consumption was approximately 12 min. The mean temperature of fried rice dishes at the consumption point was 66.1°C for cook-to-order restaurants, and 59.8°C in counterpart restaurants with a reheating process. The prevalence of *B. cereus* detected in cooked rice at the consumption point was 37.5%. Especially, 31.3% of samples were contaminated at levels higher than the microbial standard of 10^3 CFU/g specified guidelines in the Korea Food Code for *B. cereus* of 10^3 CFU/g.

The number of samples showing more than 10^5 CFU/g of ACC was 1 out of 32 (3.1%). Coliforms were detected in 21 samples out of 32(65.6%). Low heating temperature at the final cooking stage, and holding dishes for more than 2 hours at room temperature before reheating, were associated with contamination level of *B. cereus* ($P < 0.05$).

Significance: These results can be useful data for MRA studies of *B. cereus* in fried rice dishes.

P2-111 Validation of a PCR Assay for Screening Yeast and Mold for Fungal Threshold Level Testing

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Introduction: Fungi are a major cause of food spoilage. Because traditional testing can take five to seven days to complete, well validated, rapid methods for detecting yeasts and molds are needed.

Purpose: This study evaluated the inclusivity, exclusivity, and effectiveness of a PCR assay for detecting artificial fungal contamination in infant formula, yogurt and corn starch, and naturally occurring fungi in cheese, after 44 hours enrichment.

Methods: For inclusivity, 50 ATCC fungal strains, and 172 fungal isolates recovered from 435 screened foodstuffs were tested by a PCR assay for yeast and mold using the BAX® system. Thirty bacterial strains were also tested for reactivity. For method effectiveness, corn starch, yogurt, and milk-based infant formula were spiked with fungi at target levels of 0 (unspiked), 1 to 10, 10 to 50, and > 50 CFU/g. These were evaluated using the FDA-BAM method and the PCR method with five replicates per level per food type. One food type, cheese, was tested for naturally occurring fungi with 30 replicates of which 10 were positive for detectable fungi.

Results: For inclusivity testing, all 222 fungal isolates tested were found to be reactive, while the 30 bacterial strains all were non-reactive with the PCR method. For effectiveness testing, when evaluated as positive or negative at the threshold level of 10 CFU/g, results with the PCR method on all three artificially inoculated sample types were statistically indistinguishable from the reference method. For naturally contaminated cheese evaluated at a threshold level of 50 CFU/g, results with the PCR method were also statistically indistinguishable from those with the reference method.

Significance: The PCR assay was able to give plus/minus results for fungi within 48 h, providing a significant time saving which can lead to faster release of product, more useful shelf-life, and a less complicated supply chain.

P2-112 Microbiological Quality of Water Samples from Hidalgo, Queretaro and Mexico State

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Introduction: In Mexico, the health authority has a surveillance program for monitoring sources of water supply such as springs, wells and service network, as well as purified bottled water, to evaluate its quality.

Purpose: Analyze the microbiological quality of purified water and water for human consumption in the states of Queretaro, Hidalgo and Mexico.

Methods: In the period January to December 2008, 18,089 samples of water (6,125 tap water, 3004 well, 79 spring, and 8881 purified water), were analyzed for microbiological and chemical test. The determinations made were presence of aerobic mesophilic bacteria, total coliform organisms and/or fecal, as well as detection of *Escherichia coli* or *Vibrio* spp. The techniques used were established in the Mexican Official Standards.

Results: Microbiological analysis showed that of 9,475, 3,261 (22.3%) were off standard. Water from the spring showed the highest percentage of samples outside the norm (59.46). While the chemical determinations of 35,215, 8.75% were off standard. The state with the highest number of results that were off standard was the state of Mexico, with 59.37%, followed by Hidalgo with 38.55% and Queretaro with 2.09%; the latter is also the state with the fewest samples analyzed.

Significance: The results show that even when there are basic sanitation programs established, the health authorities must put more attention on chlorination practices of water network and wells, as well as strengthen health promotion activities to the population, to prevent the presence of gastrointestinal or viral diseases caused by water consumption.

P2-113 Simultaneous Separation and Detection of Multiple Foodborne Pathogens Using Magnetic Nanobeads and Quantum Dots

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Introduction: Three species of foodborne pathogenic bacteria, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium, were the major foodborne pathogens associated with poultry, meat and vegetable products. Current conventional detection methods are still limited by their time-consuming process, non-specific or non-quantitative results, complex procedures, or high cost.

Purpose: The objective of this study was to develop a rapid, sensitive and selective method for simutaneous and quantitative detection of three major pathogens in food products using magnetic nanobeads to separate and concentrate the target bacteria and quantum dots as fluorescent labels in a biosensing method.

Methods: Multi-target bacteria were separated simultaneously from samples by using magnetic nanobeads (30 nm) which were coated with specific antibodies. QDs with different emission wavelengths (525 nm, 605 nm, and 705 nm) were conjugated to anti-*Salmonella*, anti-*Listeria*, and anti-*E. coli* O157 antibodies, respectively. The nanobead-bacterial cell complexes reacted with QDs-antibody conjugates to form bead-cell-QDs complexes. The intensities of fluorescence emission peaks at 525 nm, 605 nm, and 705 nm of the final complexes were measured for quantitative detection of *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 simultaneously.

Results: Results indicated that this method could detect *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* at a concentration as low as 10 to 50 CFU/ml in food samples. Linear relationships were found between the fluorescence intensity and each of the three pathogens. The total detection time including sampling and measurement was less than

1.5 h. The result also showed that this method has the potential to be able to detect a single cell of target pathogens when a testing sample was incubated for 2 to 3 h.

Significance: This magnetic nanobeads and quantum dots based biosensing method may provide a better alternative way for rapid and efficient separation and sensitive and specific detection of foodborne pathogens for ensuring food safety and security.

P2-114 Rapid Detection of Viable *Escherichia coli* O157:H7 by Immunomagentic Separation and Light Scattering Spectroscopy

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Introduction: Escherichia coli O157:H7 continues to cause outbreaks of produce-associated foodborne illness. Contaminated water may facilitate transfer of *E. coli* O157:H7 to leafy greens during irrigation. Rapid assays should be developed to detect bacterial pathogens in irrigation water, and should be able to detect the viability of the pathogens. Light scattering spectroscopy (LSS) is a powerful technique that has been applied to qualitatively and quantitatively distinguish internal structural changes in cells upon perturbation by chemical/biological agents. When combined with bacteriophage infection of a target bacterial cell, the method can distinguish between viable and non-viable bacterial cells.

Purpose: The objective of this study was to develop a LSS based assay for rapid detection of viable *E. coli* O157:H7 in irrigation water.

Methods: Appropriate *E. coli* O157:H7 and *Salmonella* strains were seeded into individual water samples at several concentrations. 1 ml aliquots were withdrawn and subjected to immunomagnetic separation (IMS) using *E. coli* O157-specific IMS beads. Following IMS and wash steps, the beads (with any bacteria attached) were resuspended in 1 ml of lambda diluent, and one half (500 µl) of each sample was added to 10 ml of Tryptic Soy Broth (TSB) that contained 1 ml of bacteriophage AR1 (10^{10} PFU/ml). The other half of the samples were added to TSB that did not contain phage AR1, and these samples served as controls. The samples were incubated for up to 8 h. Following incubation, 100 µl aliquots were removed from each sample, and separately assayed using a light scattering spectrometer.

Results: E. coli O157:H7 was detected within 6 h in all samples that contained this pathogen. An algorithm was developed to evaluate the area under the curve of each spectra. When compared to the light scattering spectra of the non-phage treated controls, the spectra of phage infected *E. coli* O157:H7 cells differed markedly. In contrast, the spectra of samples that contained *Salmonella* were identical, due to the fact that phage AR1 does not infect *Salmonella* spp. The detection limit after 6 h of incubation was an initial concentration of 10^2 CFU/ml.

Significance: These results demonstrate the ability of LSS to detect viable bacterial cells, following phage infection. When coupled with IMS, this method may be applied to the rapid and sensitive detection of viable *E. coli* O157:H7 in irrigation water.

P2-115 Validation of a PCR Assay for Screening *Listeria* spp. in Foods and Environmental Sponges

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Introduction: Since other *Listeria* species can out-compete *L. monocytogenes* in culture, potentially leading to false negative results for the pathogenic species, some food producers are testing for *Listeria* spp. instead. Well-validated rapid methods for the detection of *Listeria* species as an indicator of possible product adulteration with *L. monocytogenes* are needed because culture-based methods take four to seven days to deliver a result.

Purpose: This study evaluated the inclusivity, exclusivity, and effectiveness of the PCR-based BAX® system approach to screening of artificially introduced Listeria in spinach, processed cheese and frankfurters, and naturally occurring *Listeria* in smoked salmon and drain sponges.

Methods: Inclusivity testing was performed at ~1 log over the claimed product sensitivity of 10^5 CFU/ml, while exclusivity testing was performed at 10^8 CFU/ml. For method effectiveness, foods were spiked with Listeria at target levels set to yield fractional positive results and were evaluated using the appropriate USDA, FDA, or AOAC culture-based method and the PCR test kit method, with twenty spiked and five unspiked samples per food type per method. Frankfurter testing was repeated at an independent laboratory. One food type, smoked salmon, and one environmental sample type, drain sponges, were tested using naturally occurring *Listeria* with twenty paired replicates.

Results: All 50 *Listeria* in the inclusivity panel were found to be reactive, while the 30 non-*Listeria* strains were non-reactive using the assay. Comparing effectiveness results for PCR and plating, results for the three inoculated sample types demonstrated Chi-square values of 0.1 to 0.46, indicating no significant difference in method performance. For the naturally occurring *Listeria* contamination of salmon and drain samples, Chi-square comparison of PCR and reference culture methods demonstrated values of 1.26 and 0.10, also indicative of indistinguishable method performance.

Significance: This data indicates that this PCR method for the detection of *Listeria* is as effective as culture-based methods while providing significant time savings.

P2-116 DSC Establishment of Enzyme Linked Immunosorbent Assay (ELISA) for Aflatoxin B1 Detection in Red Pepper Powder in South Korea

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Introduction: Red pepper powder is one of the favorite spices in Korea. Aflatoxins contaminate a wide variety of products such as foods, feeds, and spices. Among the aflatoxins, aflatoxin B1 (AFB1) is highly toxic, causing liver cancer.

Purpose: This study validated and applied a direct competitive enzyme linked immunosorbent assay (DC ELISA) to rapid detection of AFB1 in red pepper powder produced in China and South Korea.

Methods: A total of 77 red pepper powder samples were purchased from six local retailers in six cities. Pulverized samples (5g) were extracted with 70% methanol, and diluted with phosphate buffered saline. The diluent was mixed with AFB1-HRP conjugate and then added to microtiter plates coated with monoclonal antibodies. For validation of the method, the linearity of the standard curve, reproducibility and cross reactivity with AFB2, AFG1, and AFG2 were determined. Limits of detection (LOD) and quantification (LOQ) of the method were also calculated. The validated method was then used for AFB1 monitoring in red pepper powder.

Results: The method showed high sensitivity, with an analytical range from 0.01 to 1 ng/ml, and there was weak cross reactivity with other aflatoxins (AFB2: 26%, AFG1: 31%, AFG2: 23%). The method was reproducible (CV = 8%) and recoveries ranged from 84% and 102%. LOD and LOQ were 0.05 and 0.1 mg/ml, respectively. Of 77, 13 (16.9%) samples were AFB1 positive. Although additional research may be required to confirm the AFB1 positive samples by HPLC or LC/MS/MS, the validated method was fast, simple, and sensitive, with increased laboratory output.

Significance: The DCELISA can be a useful tool for presumptive screening of AFB1 residues in red pepper powder and agricultural products without a complicated cleanup.

P2-117 Study on Control of *Penicillium* spp. and Ochratoxin A in Feed by Electron-beam Irradiation
DSC

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Introduction: Penicillium spp. is widespread in Korea and all over the world on feeds and foods. They have been known to produce ochratoxin A, which is a nephrotoxic compound and has been classified by IARC as possibly carcinogenic to humans.

Purpose: The objective of this study was to determine the optimum electron-beam irradiation dose for reduction and control of *Penicillium* spp. and ochratoxin A on feeds.

Methods: Fungal spore of *Penicillium* spp. (10^7 spore/ml) was prepared in 0.85% NaCl containing 0.1% Tween 80, irradiated with electron-beam irradiation ranged from 1 to 5 kGy and then spread on potato dextrose agar (PDA). The optimal electron-beam irradiation to reduce fungal growth was estimated by checking fungal growth on PDA and OTA producibility in culture media. OTA level produced during cultivation were tested by enzyme linked immunosorbent assay (ELISA). To confirm growth inhibition of fungi on feeds by electron-beam irradiation, the feeds artificially inoculated with Penicillium spp. (10^7 spore/5 g) were prepared and irradiated with ranging from 0 to 5 kGy. To confirm the reduction of OTA by electron-beam irradiation, OTA level was analyzed by ELISA after radiation up to 5 kGy to aqueous solutions of OTA (0~10 ng/ml).

Results: Fungal spores existing in aqueous solution and on feed were completely inactivated by ≥ 3 kGy of electron-beam irradiation. No fungal growth was observed on PDA and feed. In addition, the fungal spores irradiated with ≥ 3 kGy did not produce OTA (< 0.95 ng/ml), whereas ≥ 10 ng/ml of OTA was detected in cultivation of fungal spores without electron-beam irradiation. OTA level at 10 ng/ml was reduced approximately by 78% using a minimal irradiation dose (3 kGy). The results indicate that *Penicillium* spp. and OTA on feed could be controlled by electron-beam irradiation of 3 kGy.

Significance: The results mean that electron-beam irradiation is an effective and safe treatment that can be used as a simple method to reduced fungal growth and OTA in feeds.

P2-118 One-step Immunochromatographic Strip Test for Multianalysis of Ochratoxin A and Zearalenone
DSC

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Introduction: Ochratoxin A (OTA) is a nephrotoxic compound that has been classified by IARC as possibly carcinogenic to humans and zearalenon (ZEA) is a nonsteroidal estrogenic mycotoxin. Because two mycotoxins could co-occur in food and feed, the development of a simultaneous detection assay is urgently necessary.

Purpose: This study reports the development of a one-step immunochromatographic assay for the rapid multi-analysis and on-site detection of OTA and ZEA and its application to spiked and natural corn samples.

Methods: A one-step immunochromatographic assay (OS-ICG) for the rapid multi-analysis of OTA and ZEA was developed, using optimal conditions of respective immunochromatographic assays. The effect of presence of the other toxin on the OS-ICG was investigated with each mycotoxin standards and the OTA/ZEA mixture ranged from 1.25 to 20 ng/ml. A new extraction method for the OS-ICG strip was investigated with 30% MeOH/PBS. A total of 38 corn samples were obtained, extracted and analyzed directly by the OS-ICG, and the results were compared with those of direct competitive enzyme-linked immunosorbent assay (DCELISA) and HPLC.

Results: The visual detection limits of the OS-ICG were 2.5 and 5 ng/ml for OTA and ZEA, and the results were obtained within 15 min after starting the analysis. The method showed no cross reaction to other mycotoxins. After sample preparation with 30% MeOH/PBS, the cut-off values of the OS-ICG for the spiked corn were 5 and 10 μg/kg for OTA and ZEA, respectively. In the OS-ICG analysis, OTA and ZEA were detected in 6 and 8 corn samples, respectively. Results of the OS-ICG were in good agreement with those obtained by DC-ELISA and HPLC.

Significance: The developed OS-ICG offers a rapid, easy-to-use, amd portable analytical system that can be used as a convenient qualitative tool for the on-site simultaneous determination of OTA and ZEA in cereals, food, and agricultural products in one analytical cycle.

P2-119 Validation of Enzyme-linked Fluorescent Assay for Detection of *Escherichia coli* O157:H7 in Ground and Trim Beef Samples

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Introduction: This study evaluated the limit of detection of the VIDAS *E. coli* O157:H7 ECTP, a new enzyme-linked fluorescent assay (ELFA) using phage protein capture.

Purpose: Three different sample sizes for both uncooked ground beef and trim were tested individually, and two were tested with wet compositing. The ELFA test was compared to the USDA-FSIS method, though the samples were not paired because the enrichments required different media.

Methods: There were twenty inoculated (1 to 2 CFU of *E. coli* O157:H7) replicate samples of ground beef and trim that were tested using both the ELFA and the USDA-FSIS method, along with 5 uninoculated control samples. The three sample sizes were 375 g, 75 g, and 25 g; the former two were enriched at a 1:4 ratio and the latter at a 1:10 ratio. All USDA-FSIS samples were enriched at a 1:10 ratio.

Results: Each ELFA variation was compared to the corresponding USDA-FSIS test, using a Chi-square test (not significantly different if $\chi 2 < 3.84$), but at reduced times (8 to 10 hours for the ELFA method versus 15 to 22 hours for the USDA-FSIS method).

Significance: This data shows that the new phage protein capture ELFA results were not significantly different from the USDA-FSIS results in both ground beef and trim, for the 375 g, 75 g, and 25 g sample sizes, for both individual and wet composited samples. However, the ELFA method was simpler and produced results faster.

P3-01 Effect of Packaging Materials on the Viability of Probiotic Bacteria in Goat's Milk Ice Cream DSC

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Introduction: Goat's milk and goat's milk products are attractive to some consumers due to their special nutritional and therapeutic properties. The therapeutic value of probiotic bacteria and quality of probiotic products depend on the viability of these bacteria throughout the product shelf life. Although significant packaging effects on probiotic viability of some dairy products have been observed, study of the influence of the packaging materials on viability of probiotics in ice cream has been limited.

Purpose: This study was designed to evaluate the effect of packaging materials on the viability of *Lactobacillus acidophilus*, *Bifidobacterium lactis* BB 12 and newly identified potential probiotic *Propionibacterium jensenii* 702 in goat's milk ice cream over 12 weeks of storage at -20°C.

Methods: Ice cream was produced by fermented goat's milk containing probiotic bacteria and immediately conditioned and packed in three different types of packaging: glass, polyethylene and polypropylene. Stored products were assessed for the viability of probiotics at 0, 1, 2, 3, 4, 8 and 12 weeks using spread plate techniques.

Results: All the probiotic strains were able to maintain minimum recommended levels of viability in ice cream regardless of the packaging. However, polyethylene packaging was seemed to be more effectivein retaining viability of all bacteria ($P > 0.05$). *P. jensenii* 702 counts were 8.72 log CFU/g at the beginning and were slightly decreased to 8.65, 8.59 and 8.63 log CFU/g which were stored in polyethylene, glass and polypropylene respectively. Ice cream stored in glass demonstrated highest losses of *B. lactis* BB 12 and *L. acidophilus* viabilities. The initial counts of 8.06 and 7.70 log CFU/g reduced to 7.89 and 7.49 log CFU/g respectively.

Significance: This study confirmed that different packaging materials may not have a significant effects on viability of *L. acidophilus*, *B. lactis* BB 12 and *P. jensenii* 702 in goat's milk ice cream stored for a period of 12 weeks at -20°C. Probiotic quality of this product is not adversely affected by the packaging materials due to the ability of the organisms to retain higher viability throughout the shelf life.

P3-02 Detection and Survival of *Bacillus cereus* Spores in Raw and High-temperature Short-time Pasteurized Milk DSC

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Introduction: The Food and Drug Administration's Bacteriological Analytical Manual recommends two different methods of enumeration for *Bacillus* spp.: (1) standard plating method using mannitol egg-yolk polymyxin (MYP) agar and (2) most probable number (MPN) method with Tryptic Soy Broth supplemented with polymyxin sulfate.

Purpose: The purpose of this study was to determine whether MYP or MPN method is better at detecting and enumerating *Bacillus* spp. in raw and pasteurized milk.

Methods: Preliminary research evaluated the use of Microbiologics EZ-Spore™ technology for the preparation of a *B. cereus* inoculum. To obtain an inoculum level of approximately 3.6 logs, the best procedure consisted of dissolving 5 ml of *Bacillus cereus* EZ-Spores in distilled water for 30 min. Raw and pasteurized skim, 2%, and whole milk were inoculated with *Bacillus cereus* EZ-Spores along with non-inoculated raw milk, held at 4°C, sampled at 0, 24, 48, 72, and 96 h, and evaluated for *B. cereus* using MYP and MPN enumeration methods.

Results: Initial inoculum level was 0.6 and 1.8 log CFU/ml for MYP and MPN, respectively. No *B. cereus* colonies were observed on MYP for any of the milk samples. For the MPN method, population averages ranged from 2.7 to 3.6, 2.4 to 3.7, 2.4 to 3.0, 2.5 to 3.4, and 3.2 to 3.4 log CFU/ml for raw, non-inoculated raw, pasteurized skim, 2%, and whole milk samples, respectively, over the five sampling times. Although populations increased over time, no significant differences ($P > 0.05$) were observed among populations during the five sampling periods. A significance difference ($P < 0.001$) was observed between the two methods of enumerating *B. cereus* in milk.

Significance: According to this study, the MPN method is better for enumerating *Bacillus cereus* in milk than plating on MYP. Even though the inoculum level of 3.6 was achieved, better methods of *Bacillus* spp. spore production should be studied further.

P3-03 Thermal Resistance of *Listeria monocytogenes* Scott A in Concentrated Ultrafiltered Milks and Reconstituted Milk Powder Related to the Effect of Different Milk Components

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Introduction: Pasteurization parameters for standard milk are well established and set by regulation (PMO). However, as solids levels increase, an increased amount of heat is required to destroy any pathogens present. This effect is not well characterized. Despite this shortcoming, innovations in food processing have led to a desire to create liquid products with higher levels of solids.

Purpose: To determine the effect of increased dairy solids levels on the thermal resistance of *L. monocytogenes* in milks through the use of ultrafiltered milk, reconstituted milk powder, milk and the milk components fat, lactose, and caseinate.

Methods: L. monocytogenes was cultured, harvested, and resuspended in ultrafiltered milk, reconstituted milk powder, and the milk components of differing concentrations. Samples were sealed into capillary tubes, heated at different temperatures for varying times and enumerated to evaluate survival.

Results: Increased lactose and caseinate concentrations did not result in increased thermal resistance. In addition, the level of milk fat, up to 10 % of the total solids in ultrafiltered whole milk, did not increase thermal resistance when compared to ultrafiltered skim milk. Reconstituted skim milk powder at higher concentration (27% total solids D62 = 1.26 +/- 0.19 min, z = 5.70) did result in increased thermal resistance ($P < 0.05$), as compared to reconstituted skim milk powder at 17.5 % (D62 = 0.82 +/- 0.08 min, z = 5.57) and ultrafiltered whole milk at 27% total solids (D62 = 0.68 +/- 0.08 min, z = 5.04).

Significance: It was demonstrated that total solids could not be used as the sole predictor of increased thermal resistance through the range tested. Increases in thermal resistance measured were most likely due to the presence of salts or the combination of salts with other milk components. Other components of milk, such as fat, did not increase thermal resistance.

P3-04 Use of Nisin and Caprylic Acid to Control *Listeria monocytogenes* in Queso Fresco

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Introduction: Listeria monocytogenes is a foodborne pathogen responsible for outbreaks due to consumption of contaminated fresh Hispanic cheeses. "Queso fresco" is the most popular fresh Hispanic cheese, but it may be susceptible to contamination and growth of *Listeria* since it is preserved only by refrigeration.

Purpose: The objective of this research was to determine the inhibitory effect of caprylic acid (CA) and nisin additions on the population of *L. monocytogenes* in queso fresco and the impact of those additions on the microbial quality and sensory characteristics.

Methods: Batches of queso fresco curds were inoculated with mixtures of six *L. monocytogenes* strains, mixed with nisin (0.5 g/kg as Nisaplin®) and CA (0.8 to 3.2 g/kg), molded, packed and stored at 4°C for 3 weeks. Counts of *L. monocytogenes* were obtained on PALCAM agar plates. Aerobic plate count (APC), lactic acid bacteria (LAB) and psychrotrophic bacteria (PB) counts were determined by standard methods. A 3-alternative forced choice test (3-AFC) protocol with 31 volunteers was used to detect sensory differences between samples, and 67 Hispanic consumers rated their liking of the cheeses.

Results: Combinations of nisin and caprylic acid rapidly reduced the initial count (3.5 log CFU/g) of *L. monocytogenes* in cheese samples to almost undetectable levels (< 2 log CFU/g) and its count rarely reached 2 log CFU/g or greater during storage. Cheese with nisin and CA had significantly less APC and LAB counts than control samples. Panelists distinguished differences between treated and control samples, but those differences did not appear to have a marked impact on acceptance by queso fresco consumers.

Significance: These results indicated that combinations of nisin and CA can be effective treatments to control *L. monocytogenes* in queso fresco, with little impact on product quality. This treatment may contribute to prevention of future listeriosis cases.

P3-05 Evaluation of 3M™ Petrifilm™ Aerobic Count Plate for Enumerating Psychrotrophic Microorganisms in Dairy Products

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Introduction: Traditional methodologies used for enumeration of psychrotrophic microorganisms in food are time consuming, usually taking up to 10 days. There are very few studies suggesting the reduction of this time by increasing incubation temperatures; however, one of these suggestions, found at American Public Health Association [APHA], uses the combination of 25 h at 21°C for milk and cream. 3M™ Petrifilm™ Aerobic Count [AC] Plates have been designed to be used in the enumeration of mesophilic microorganisms, but to date, there has been little published data for enumeration of psychrotrophs.

Purpose: This study was initiated to evaluate the efficacy of incubation for 25 h at 21°C for the enumeration of psychrotrophs in naturally contaminated dairy product samples, and to verify the usefulness of the AC plate under these conditions.

Methods: Seventy-four dairy product samples (cheeses, raw and pasteurized milk) were simultaneously analyzed using three methods: APHA recommendations for psychrotrophs – Plate Count Agar [PCA] at 7°C/10 days; PCA at 21°C/25 h and 3M Petrifilm AC plates at 21°C/25 h. The results were compared, submitted to Kruskal-Wallis test (Minitab® Release 14.20 Statistical Software) and linear regression. For statistical purposes, only samples showing <1 log CFU/ml by all three methods were considered equal to 1 log.

Results: Results analyzed using Kruskal-Wallis test and linear regression showed that counts between traditional media (PCA) using both incubation conditions were not statistically different ($P > 0.05$; $R^2 = 94.5\%$). The R^2 for the comparison between AC plates and PCA at 21°C/25 h and 7°C/10 days were 95.9% and 92%, respectively; and the *P*-value was > 0.05 for both.

Significance: AC plates show promise as an alternative method to the traditional enumeration of psychrotrophs in dairy products, when 21°C/25 h is used. These findings would help the dairy industry obtain results in a more more easily and timely manner. Additional studies to verify these conclusions and application are recommended.

P3-06 Survival Characteristics of Persistent Dairy *Salmonella* Strains

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Introduction: Salmonella contamination is of concern to the dairy industry, particularly in the hygienic dry milk powder factory environment. Specific *Salmonella* strains persist in these environments and may be reisolated over months or years, resulting in recontamination of the product. These persistent strains may have characteristics which allow them to better survive in these environments.

Purpose: To compare some survival characteristics of persistent *Salmonella* strains from dairy environments to those of transient strains from the same environment and of strains from other environments.

Methods: A total of 4 persistent dairy *Salmonella* strains, 3 transient dairy strains and 4 non-dairy strains were compared. Hydrophobicity of strains was determined using hydrophobic interaction chromatography and bacterial adhesion to hydrocarbon (xylene) methods. Desiccation survival was tested by drying strains in milk under low relative humidity for 7 days, with enumeration on nutrient agar before drying and after drying. Survival of strains in the presence of a sanitizer was determined by enumerating previously desiccated cells pre- and post-exposure to chlorine (13%) on nutrient agar.

Results: No differences were apparent between strains with respect to hydrophobicity or desiccation only, other than for two of the transient strains. Specifically, a single *S.* Sofia strain was significantly ($P < 0.05$) more hydrophobic (35% versus ~6% attachment) than the rest of the isolates, while another *S.* Sofia strain was significantly ($P < 0.05$) more sensitive to desiccation (5 log reduction vs ~ 3 log reduction) than the rest of the strains. More importantly, however, a persistent dairy strain, *S.* Anatum 1653a, survived sanitizer exposure after desiccation (< 0.2 log reduction versus 3.5 log reduction) significantly ($P < 0.05$) better than all other strains.

Significance: This study suggests that desiccation and sanitizer resistance in *Salmonella* strains may account for their persistence in milk powder factories and that alternative measures are required to control them.

P3-07 Isolation and Identification of Microorganisms Responsible for Ropy Milk

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Introduction: Ropy milk is a quality defect that is occasionally reported in raw and pasteurized milk. Anecdotal reports suggest that the causative microorganisms either are not eliminated during high-temperature short-time (HTST) pasteurization or cannot be controlled in packaged, pasteurized milk containers that are cleaned with typical cleaning and sanitation procedures.

Purpose: To isolate and identify microorganisms responsible for ropy milk.

Methods: Raw and pasteurized milk samples identified as ropy through quality checks by four United States manufacturing facilities were sent to the Food Research Institute for testing. Samples were plated directly and after heat shock (80°C, 10 min) on plate count agar, Tryptic Soy Agar with 5% sheep blood, MRS lactobacilli agar, KF *Streptococcus* agar and crystal violet tetrazolium agar, and incubated at 22 and 7°C for 2 to 3 and 7 days, respectively. Purified isolates were tested for their ability to cause ropiness in 2% and skim milk at 22 and 7°C. Ropy isolates were identified using Gram staining and biochemical analysis (Remel RapID and MicroID strips). Pulsed-field gel electrophoresis (PFGE) was used to characterize select isolates of the same species from multiple sources. Thermal inactivation at 78°C was also evaluated for several isolates.

Results: Four hundred isolates were recovered from 48 pasteurized or raw milk samples over a six month period; 38 isolates were able to cause ropiness when inoculated to pasteurized milk samples. *Streptococcus constellatus* and *S. intermedius* were the most common species isolated from both raw and pasteurized samples. Thermal resistance testing of *S. constellatus* and *S. intermedius* at 78°C yielded a 5 log reduction within 5 s; therefore the organisms should be eliminated from raw milk during HTST. PFGE performed on several *Streptococcus* isolates demonstrated that isolates from raw milk were different from those isolated from pasteurized milk.

Significance: This study has identified *S. constellatus* and *S. intermedius* among the primary microorganisms responsible for ropy spoilage of milk. Future studies will determine if the microflora shifts seasonally, and additional PFGE analysis and thermal resistance testing will be used to identify when the organisms contaminate the milk and to develop control strategies.

P3-08 Susceptibility of Desiccated Enterobacteriaceae to Chlorine, Heat and Spray Drying

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Introduction: Enterobacteriaceae are persistent in dry dairy factory environments and may enter powdered milk and infant formulae products from this source.

Purpose: To determine if desiccation and environmental humidity influence the resistance of Enterobacteriaceae to sanitiser, heat and spray drying treatments.

Methods: UHT skim milk with added *Salmonella*, *Cronobacter* (*E. sakazakii*) or *E. coli* was dried onto surfaces and stored at 20°C in an atmosphere of 33 and 85% relative humidity (RH). After one and eight days, the dried preparations were rehydrated and exposed to sodium hypochlorite (25 ppm) or heat (56°C) for one min. Desiccated *Cronobacter* (33% RH, 8 days) was also added to milk concentrate, spray dried and stored at 20°C. Undesiccated cells in UHT skim milk were used for controls. Survivors were determined by viable count on Tryptone Soya Yeast Extract Pyruvate Agar.

Results: Storage humidity did not influence susceptibility of desiccated *Salmonella* and *Cronobacter* to sodium hypochlorite ($P > 0.05$), but *E. coli* was more resistant after storage at 85% than at 33% RH ($P < 0.05$). *Salmonella* and *Cronobacter* were more sensitive to heat after storage at 85% than at 33% RH ($P < 0.05$), while the converse occurred for *E. coli*. The resistance of the isolates to sodium hypochlorite and heat was not increased by desiccation. Desiccated *Cronobacter* cells survived spray drying and storage better than undesiccated cells. After 36 weeks storage, the milk powder counts had decreased by 1.9 and 4.0 log CFU/g for the desiccated and undesiccated cell treatments respectively (compared with concentrate counts adjusted for milk solids).

Significance: Understanding the impact of desiccation and environmental humidity on inactivation treatments and processing is important for the development of intervention programs for Enterobacteriaceae to ensure the manufacture of safe powdered milk and infant formula products that meet microbial specifications.

P3-09 Survival of Enterohemorrhagic and Avian Pathogenic *Escherichia coli* from Spinach Plants after Overhead Irrigation with (Currently Acceptable) Contamination Levels

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Introduction: In response to the growing food safety issues surrounding fresh cut lettuce and other leafy green commodities in the US, the Leafy Green Marketing Agreement (LGMA) proposes new commodity-specific guidelines that encompass all steps on the production-to-distribution continuum to ensure the safe on-farm production, processing and distribution of these commodities. Many of the proposed guidelines are based on metrics from current scientific literature, while some defer to standards not intended for agricultural use. For example, the current guidelines for *E. coli* levels in irrigation water was set by the EPA for recreational water use at ≤ 126 MPN/100ml (rolling geometric mean n = 5, and any single sample must be ≤ 235 MPN/100ml).

Purpose: This study examined the irrigation water standards stated in LGMA.

Methods: Avian Pathogenic (APEC) and Enterohemorrhagic *E. coli* O157:H7 (EHEC) isolates, resistant to nalidixic acid, were adapted for growth in bovine manure and used to prepare two irrigation solutions in sterile water. Three irrigation solutions (APEC: 156 CFU/100 ml; EHEC: 77 CFU/100ml; water control) were sprayed for 25 s each (1.5ml delivered) onto individual mature spinach plants using an air-brush to simulate overhead irrigation. Plant tissue was harvested daily for three days, and enrichment techniques and real-time PCR were used to detect virulence factors of EHEC (*stx*2) and APEC (espA).

Results: Real-time PCR results suggest that very low levels of EHEC were, in fact, delivered to the surfaces of the spinach plants. No enrichments were able to recover viable *E. coli* cells.

Significance: Low levels of *E. coli* in irrigation water can be recovered from spinach plants by real time PCR methods. It is unclear how long *E. coli* cells are viable on spinach leaves. This study provides preliminary data to support the current LGMA guidelines for acceptable *E. coli* levels in irrigation water that contacts edible portions of the crop.

P3-10 The Effect of Storage Conditions on the Behavior of *Escherichia coli* O157:H7 and Normal Microflora on Packaged Fresh Spinach

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Introduction: Outbreaks of *E. coli* O157:H7 (EC) infection have been linked to fresh leafy greens such as spinach. Temperature and package atmosphere affect the microbiological quality of spinach, and these factors also may be able to minimize the risk of EC in the product.

Purpose: The objective was to determine temperature and package atmosphere effects on behavior of EC and normal microflora in packaged spinach.

Methods: Micro-perforated and non-perforated packaging films for retail (20% O_2 / 3% CO_2) and low-oxygen (0% O_2 / 15% CO_2) conditions, respectively, were used for model packages containing 100 g spinach. EC was spot-inoculated on leaves (100 CFU/g), and the package was sealed, vacuumized, and injected with appropriate volumes of O_2, CO_2, and N_2 to obtain the targeted atmosphere. Packages were stored at 5 or 12°C for up to 8 days. Triplicate samples were assayed for EC and normal microflora, including mesophiles (ME), psychrotrophs (PS), yeasts and molds (YM), lactic acid bacteria (LA) and Enterobacteriaceae (EN).

Poster

Results: Normal microflora in several batches of freshly-harvested spinach ranged as follows: 4 to 7 (ME), 5 to 7 (PS), 3 to 5 (YM), 0.5 to 2 (LA), and 4 to 6 (EN) log CFU/g. In 20% O_2/3% CO_2, normal microflora increased 1 to 2 log compared to initial levels, whether stored at 5 or 12°C during the 8-day storage period, while EC decreased by 0.5 to 1.5 log. In low oxygen, normal microflora levels remained unchanged at 5°C, while EC declined by 1 log. However at 12°C, EC levels remained stable or increased by approximately 0.5 log. The higher temperature and low oxygen conditions allowed increased survival of the pathogen, compared to survival at lower temperature and high oxygen conditions.

Significance: Temperature and package atmosphere affect survival of *E. coli*. Other conditions may also minimize pathogen risk in packaged spinach.

P3-11 DSC Thermal Resistance of Heat-shocked *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in Dairy Compost

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Introduction: Proper composting, is critical for inactivation/killing of pathogens in animal wastes. However, persistence and survival of pathogens during composting has been reported.

Purpose: To study thermal inactivation of heat-shocked *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* in dairy compost.

Methods: Three strain mixture of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* were heat-shocked at 47.5°C for 1 h, and then inoculated into the finished dairy compost (ca. 10^7 CFU/g). The non-heat-shocked cultures were served as control. Compost packed in pouches were placed in environmental chamber set at 50, 55 and 60°C with ca. 70% humidity. The surviving population of each species was analyzed in duplicate by direct plating or enrichment at predetermined time excluding the come-up time. The inactivation kinetic was modeled by mixed Weibull model.

Results: The heat-shocked *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* survived better ($P < 0.05$) at 50°C with log reduction of 2.67, 3.21 & 3.89 within 4 h in comparison to control cultures of 3.55, 4.77 and 5.09 log reduction, respectively. The heat-shocked cultures had 1.17, 1.85 and 2.1 log reduction within 1 h at 55°C for *E. coli* O157:H7, *Salmonella* and *L. monocytogenes,* respectively, whereas control cultures had 3.98, 5.6 & 4.7 log reductions, respectively. At 60°C, bacterial reduction was significant during the come-up time of 14 min between control and heat-shocked cultures of all three species. *L. monocytogenes* at all three temperatures exhibited extensive tailing in the survival curves. The mixed Weibull distribution model fitted well the survival curves of pathogens, with differences in the shape parameter of heat-shocked and control cultures.

Significance: Our results suggest that heat-shock may extend the survival of pathogens at lethal temperature during composting and thereby promote survival through the composting process, which has the potential to result in contamination of fresh produce and the environment.

P3-12 DSC Evaluation of Physical Coverings Used to Reduce *Escherichia coli* O157:H7 Populations at the Surface of Compost Heaps

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Introduction: Minimal maintenance of compost heaps, including no heap turning, may result in extended survival of pathogens, as material near the compost surface lacks exposure to elevated temperatures.

Purpose: The goal of this study was to apply physical coverings to control *Escherichia coli* O157:H7 at the surface of dairy composting heaps under field conditions.

Methods: Two composting trials were conducted in the spring and summer seasons, each with four compost heaps composed primarily of a cow manure-sawdust mixture. Compost samples were inoculated with avirulent *E. coli* O157:H7 B6914 and packed into either Tyvek® bags or vented polystyrene trays and secured to the heaps. Two compost heaps were bisected; samples bags were placed at the east and west sections of each compost half and then covered with finished compost with 30 or 50% moisture at 15 or 30 cm thicknesses. Another heap was covered with ca. 13 cm of fresh hay, and the remaining heap was left uncovered, serving as the control. Populations of *E. coli* O157:H7 and Enterobacteriaceae were enumerated or enriched over pre-determined intervals.

Results: Ca. 7 log CFU *E. coli* O157/g compost in the heap covered with 15 or 30 cm of finished compost (30% moisture) was eliminated within 21 and 5 days in composting trials 1 and 2, respectively. With other coverings, the pathogen was below detectable limits after 60 and 5 days of composting, in trials 1 and 2, respectively. In both trials, *E. coli* O157:H7 was detected in the control (uncovered) treatment through day 120. Within week one of composting, maximal temperatures at the interface between compost surface and compost coverings were achieved, ranging from 41 to 48°C in trial 1 and 47 to 50°C in trial 2.

Significance: Our results demonstrated that covering compost heaps with finished compost or hay can expedite pathogen inactivation at the surface of heaps, thereby reducing the probability of environmental contamination.

P3-13 DSC Interaction of *Escherichia coli* O157:H7 with Growing Spinach Plants

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Introduction: In recent years, there has been increasing incidence of foodborne outbreaks linked to fresh and fresh-cut fruits and vegetables in the United States. Particularly noteworthy was the 2006 *Escherichia coli* O157:H7 outbreak associated with pre-bagged baby spinach. To better ensure produce safety, research is urgently needed to fill critical knowledge gaps, especially regarding the route and mechanism of contamination.

Purpose: The study aimed to determine whether *E. coli* O157:H7 would be present in the aerial leaf tissue of a spinach plant when introduced via soil inoculation at different growth stages in a greenhouse setting.

Methods: Spinach seeds of a standard commercial variety were sown individually in 8-inch pots and watered daily and fertilized weekly after germination. Soil inoculation at two levels (10^8 and 10^4 CFU/ml) of an *E. coli* O157:H7 green fluorescent protein (GFP)-expressing strain was conducted on a weekly basis for a total of five times after germination. Inoculated spinach samples were harvested weekly and examined for the presence of *E. coli* O157:H7 on leaves and in surrounding soil.

Results: Among 60 plant samples examined for internal leaf contamination, only one plant yielded positive for the GFP-expressing *E. coli* O157:H7 strain. Surface contamination occurred occasionally and clustered between three to five weeks after germination. No surface contamination occurred among leaves younger than three weeks of age. On the other hand, when inoculated at 10^8 CFU/ml, the GFP-expressing *E. coli* O157:H7 survived the entire cultivation period but with gradually reduced levels.

Significance: The experiments demonstrated that the internalization of *E. coli* O157:H7 into a growing spinach plant under greenhouse conditions was a rare event but that contamination did occur, primarily when the plants reached 3 weeks of age. The study provided important data to further assess the association between spinach age and the potential contamination with *E. coli* O157:H7.

P3-14 Fate of Internalized *Escherichia coli* O157:H7 on Field Grown Spinach Treated with Contaminated Irrigation Water

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Introduction: Internalization of *Escherichia coli* O157:H7 into edible portions of leafy green plants has been reported under greenhouse conditions, but whether internalization occurs under field conditions and the associated contributing factors need to be studied.

Purpose: To determine the fate of *E. coli* O157:H7 applied at varying concentrations to spinach fields through drip or spray irrigation water and the influence of plant growth stage on surface and internalized contamination of leaves and roots.

Methods: A four strain mixture of Shiga toxin-negative *E. coli* O157:H7 was added to irrigation water to give concentrations of 10^2, 10^4, or 10^6 CFU/ml. The water was administered once to the soil at the base of early-, mid-, or late-season spinach plants as well as to spinach plants in mid- and late-season through an overhead spray. Soil and plants (roots and leaves) were sampled up to 3 weeks following exposure and analyzed using either direct plate count or enrichment culture. A mercury chloride/ethanol wash of plant tissue prior to grinding and analysis targeted internalized pathogen.

Results: E. coli O157:H7 persisted in soil 21, 22, and 7 days following application of contaminated irrigation water to soil in the early-, mid-, and late-growing seasons. Despite persistence in soil, internalized pathogen was never detected in spinach leaves nor in root samples collected up to 3-weeks following the early- or late-season exposure. For plants exposed to contaminated soil mid-season, 5 of 30 root samples collected 7-days post-exposure were positive for internalized *E. coli* O157:H7. Following spray inoculation, the pathogen could only be detected in or on leaves through enrichment culture on the day of inoculation in mid- and high-dose treated spinach plants. Seven days post-spraying, all spinach leaves tested negative for surface or internal contamination.

Significance: This data will be useful in risk assessment studies involving contamination of leafy green fields with contaminated irrigation water.

P3-15 The Survival of *Escherichia coli* O157:H7 in Cucumber Fermentation Brines

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Introduction: Bacterial pathogens have been reported on fresh cucumbers and other vegetables used for commercial fermentation. Fermented vegetables have an excellent safety record, and are exempt from acidified food regulations. However, there is little data showing how bacterial pathogens are killed in cucumber fermentations.

Purpose: Lactic acid bacteria produce a variety of antimicrobial metabolites, and recycled brines are commonly used for commercial fermentation. The objective was to determine how acid resistant *Escherichia coli* O157:H7 strains are killed in these brines.

Methods: We obtained brine samples from six 40,000 L commercial cucumber fermentation tanks at different stages of fermentation. The brines were sterilized by filtration and inoculated with a cocktail (10^6 CFU/ml) of *E. coli* O157:H7 strains prepared to induce acid resistance. Brine composition was determined by HPLC. Inoculated brines were incubated anaerobically at 30°C. The surviving cell numbers were determined on non-selective media using a spiral plater and automated plate reader.

Results: Brines contained up to 50 mM sugar, 30 to 150 mM lactic acid, 4 to 8 percent NaCl, and had pH values of 3.0 to 4.2. Most samples contained sorbic acid (< 0.04 %), used to inhibit spoilage microorganisms in commercial fermentations with air purging. In a brine sample with 55 mM sugar, 32 mM lactic acid, 0.02% sorbic acid and pH 4.1, the cell numbers of *E. coli* decreased one log after seven days, although by 15 days there were no detectable cells (< 10^2). Most samples had no detectable cells after seven days. There was no apparent change in acid or sugar concentrations or pH in any of the samples during incubation, indicating there was no growth of the *E. coli* strains.

Significance: These results indicate that a typical fermentation time of two or more weeks may be sufficient to assure the destruction of pathogenic *E. coli* strains in fermentation brines, independent of active bacterial competition.

P3-16 Pre-harvest Internalization of *Escherichia coli* O157:H7 into Lettuce Leaves as Impacted by the Presence of Insects

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Introduction: Environmental pests serve as reservoirs and vectors of zoonotic pathogens; however, it is unknown whether insect pests, through their feeding activities, could also redistribute the pathogen on contaminated leaves to internal sites of plant tissue.

Purpose: To differentiate the degree of tissue internalization of *E. coli* O157:H7 applied at various concentrations to lettuce leaves (abaxial or adaxial side) and subjected to mechanical abrasion; and to ascertain whether insects that commonly infest lettuce could stimulate internalization of surface-contaminated pathogens.

Methods: An inoculum mixture of gfp-labeled *E. coli* O157:H7 (5 isolates) at 10^6 to 10^8 CFU/ml was applied (25 µl) as a fine mist or as fine drops to either the abaxial or adaxial surface of lettuce leaves and analyzed after potted plants had benn held for 48 h within an environmental growth chamber. Immediately after drop inoculation, a rounded glass rod was rubbed very gently over the surface. In a separate experiment, leaves were exposed for 24 h to one of 4 insect types (5 cabbage loopers, 10 aphids, 10 thrips, or 15 whiteflies) either prior to or following leaf inoculation. For each trial, a portion of inoculated leaves were analyzed for total populations of *E. coli* O157:H7 while the other portion received a surface disinfection treatment (ethanol/$HgCl_2$) prior to grinding the tissue and analyzing for internalized pathogen populations.

Results: No internalization of *E. coli* O157:H7 occurred when leaves were exposed to a 10^6 CFU/ml spray. Pathogen internalization did occur when exposed to a 10^8 CFU/ml spray, and higher incidences were noted for contaminated abaxial (underside) compared to adaxial (topside) leaves and leaves subjected to mechanical abrasion. Exposure of surface-contaminated leaves to insects reduced the number of leaves having internalized *E. coli* O157:H7.

Significance: Understanding the conditions that contribute to pathogen internalization and survival within leafy greens will be valuable in developing pre-harvest management practices for producing a safe product.

P3-17 Internalization of Enteric Viruses in Spinach and Green Onions

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Introduction: There have been several outbreaks of hepatitis A virus (HAV) linked to the consumption of fresh produce, including strawberries, lettuce and green onions. Sources of viral contamination of produce remains unclear; however, contaminated water, soil, flooding fields and wildlife are likely to contribute. HAV has been shown to be internalized in hydroponically grown green onions.

Purpose: This study addressed pre-harvest routes of enteric virus introduction into fresh produce. Virus survival in soil and internalization into growing spinach and green onion plants through contaminated soil and water were investigated.

Methods: Spinach and green onion seeds were planted and grown in the University of Delaware greenhouse. Ten days post planting, plants were transferred to growing pots with soil or water contaminated with 2×10^7 log TCID50/g HAV. Days 5, 10 and 20 post-inoculation plant samples were collected, washed with Virkon to remove external viruses present and homogenized in Na_2HPO_4 (pH 9.5) to release internal virus particles. Both plant and soil samples were analyzed by TCID50 and qPCR.

Results: Internalized HAV was detected by qPCR on day 5 post-inoculation in one spinach plant contaminated through inoculated soil. This spinach plant contained 2.43 logs PCR units/mL of HAV internally; however, HAV was not detected in this spinach plant through TCID50 analysis. HAV was undetectable in all other plant and soil samples between days 5 and 20, which could be due to adsorption of the virus particles to the soil. Viral recovery and survival in soil will be further investigated.

Significance: In spinach and green onions grown under natural conditions and exposed to a single inoculation of contaminated water, HAV did not internalize into plants according to results with current evaluation techniques. Contaminated soil produced HAV internalization in one plant; however, after 5 days post inoculation, HAV was not detectable.

P3-18 Effect of Heat and Drought Stress during Growth of Lettuce (*Lactuca sativa L.*) on Internalization of *Escherichia coli* O157:H7

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Introduction: Outbreaks of *E. coli* O157:H7 associated with lettuce and spinach have raised questions regarding the potential for internalization of *E. coli* O157:H7 in vegetables, especially when grown under stressful conditions such as high temperature, drought, or waterlogging.

Purpose: The objective of this study was to determine if heat stress during growth of lettuce under different water conditions in soil caused internalization of *E. coli* O157:H7 in leaves or roots.

Methods: Iceberg and Romaine lettuce were grown in sandy soil in an environmental chamber at 23°C during the day and 7°C at night. Thirty days after transplantation, potting soil with lettuce was inoculated with a 5-strain mixture of *E. coli* O157:H7 at 10^4 and 10^6 CFU/g soil. The lettuce plants were exposed to two temperature regimes, i.e., (1) 36°C during the

day and 15°C at night for 2 days; (2) 32°C during the day and 15°C at night for 3 days. Control plants were grown at 23°C during the day and 7°C at night. Roots, leaves and soil samples were collected immediately after receiving the heat stress treatments and assayed for *E. coli* O157:H7.

Results: After enrichment culture, all inoculated soil and rhizosphere samples from inoculated soil were positive for *E. coli* O157:H7. All leaf surfaces and ground leaves after surface disinfection were *E. coli* O157:H7-negative. After surface disinfection, all ground roots, except for one, were *E. coli* O157:H7-negative. Slightly less *E. coli* O157:H7 populations were detected in soil samples held under heat stress than in those from inoculated control soil.

Significance: Heat stress during growth of lettuce under different soil moisture conditions did not cause internalization of *E. coli* O157:H7 in Romaine or iceberg lettuce plants.

P3-19 Surface Water Irrigation Disinfection in Lettuce Production

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Introduction: Irrigation water has been implicated in a number of produce-related foodborne illness outbreaks, most recently with *Salmonella* Saintpaul on fresh market tomato and pepper.

Purpose: In this study, we examine the use of calcium hypochlorite delivered via the PPG Accutab® Chlorination system to treat agricultural surface water used for drip and sprinkler irrigation.

Methods: Lettuce beds were prepared using standard methods at the University research farm. Irrigation was applied through either the use of drip tape under black plastic mulch or sprinklers (overhead). In the overhead irrigation portion of the study, drip irrigation was used to apply fertilizers to the developing plants and to eliminate the possibility of confounding results due to fertilizers contacting developing leaves. Treatments included overhead irrigation with and without chlorination and drip irrigation with and without chlorination, for a total of four unique treatment combinations. Each treatment was replicated three times. Fecal coliform levels in the irrigation water and on the leaves were tested with either a ten-tube or three-tube MPN as outlined in the FDA Bacterial Analytical Manual. Free chlorine levels were monitored at the furthest end of the treatment application (either the last drip tape or the furthest sprinkler) and were maintained at a level of 1 to 3 ppm free chlorine.

Results: While the fecal coliform levels in the water were low, the levels reached > 1600 MPN/100 mL during the season in the non-chlorinated irrigation water. The levels in both chlorinated systems remained < 1.8 MPN/100mL.

Significance: The results suggest that this system is effective in controlling irrigation water contamination. While fecal coliforms where monitored on the leaves, their levels were low, and the only discernible contamination did not correlate to irrigation method or chlorination. These studies will be repeated in the 2009 growing season, using tracer organisms to follow the transfer rates from water to lettuce.

P3-20 Effect of Sodium Hypochlorite and High Power Ultrasound on *Escherichia coli* O157:H7 in Lettuce Homogenate and on Romaine Lettuce

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Introduction: The 2006 *E. coli* O157:H7 outbreak in spinach caused great concern over the safety of leafy greens, and improving product safety became a priority. Sodium hypochlorite is a sanitizer widely used by the fresh produce industry to reduce microbial contamination in produce wash water. The incorporation of a non-thermal process such as high power ultrasound (HPU) during produce washing may be one way to enhance the safety of leafy greens.

Purpose: The objective was to determine whether the use of sodium hypochlorite in combination with HPU increases the reduction of *E. coli* O157:H7 in lettuce homogenate and on the surface of lettuce.

Methods: Two strains of *E. coli* O157:H7 were used separately for the homogenate and inoculated lettuce tests, and experiments were performed in duplicate at 10 and 20°C. *E. coli* O157:H7 suspended in lettuce homogenate (~8 log) was added at 1:10 (v/v) to 25-200 ppm sodium hypochlorite and was treated with or without HPU for 15, 30, 60 or 120 s and the sanitizer was neutralized. Romaine lettuce pieces (5 cm^2) were spot inoculated with 100 µl *E. coli* O157:H7 (~7 log), dried then immersed in 700 ml sodium hypochlorite (50 to 200 ppm) with or without HPU for 120 s.

Results: An additional 0.5 to 1 log reduction with HPU at both 10 and 20°C was observed when *E. coli* O157:H7 was suspended in lettuce homogenates and treated with 100 ppm sodium hypochlorite for 120 s. Further reductions in *E. coli* counts were observed at 150 and 200 ppm sanitizer with HPU. Treating inoculated lettuce pieces with 50 ppm sodium hypochlorite at 10°C provided an additional 1.5 log reduction when HPU was used. At 20°C with HPU, an additional reduction (2 log) was seen with 200 ppm sodium hypochlorite.

Significance: The results suggest that HPU in combination with an appropriate sanitizer may enhance removal or inactivation of *E. coli* O157:H7 during washing of romaine lettuce.

P3-21 Microbial Contamination of Spinach Placed in Close Proximity of Cattle Feed Yard Operations

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Introduction: In the fall of 2006, there was a major outbreak of illnesses caused by *E. coli* O157:H7 linked to spinach. Trace back investigations resulted in identification of the origin of contaminated product, but the mode of contamination was not determined. The place of source of contamination was identified as California, where a great deal of produce is cultivated in close proximity to cattle feed yards. Spinach is exposed to several environmental factors throughout the growing process that can cause contamination.

Purpose: The objective of this study was to determine if exposure to feed yard dust can cause an increase in the total number of indicator organisms (generic *E. coli*) in spinach plants.

Methods: Spinach bundles were obtained from local grocery store and tested for background microflora. Exposed bundles were set up in an upright position at three locations (0, 20 and 50 yards) from the loading area in the direction of wind. Similarly, a second set of bundles were set under plastic covering at each location, right beside the exposed ones, to serve as a control. Sampling was conducted at 6, 12 and 24 h and total numbers of generic *E. coli* were determined by use of petrifilm, according to manufacturer's recommendations, as an indicator of pathogen contamination. A total of four replications were conducted.

Results: There was a significant increase in generic *E. coli* numbers from 0 logs at 0 hours to 1.90, 2.47 and 2.49 logs over 6, 12 and 24 h of exposure, respectively, at 0 yard location. There was a significant increase from 0 logs at 0 hours to 1.85, 2.04 and 2.48 logs over 6, 12 and 24 hours of exposure, respectively, at the 20 yard location. There was a significant increase from 0 logs at 0 hours to 1.71, 2.28 and 2.29 logs over 6, 12 and 24 hours of exposure, respectively, at the 50 yard location. None of the control samples had any generic *E. coli* numbers throughout the sampling.

Significance: Data indicate that the cattle feed yard may potentially contaminate vegetable crops through dust if the crops are in close proximity. It is important to determine pathogen carriage in the dust and the potential for the dust to cause actual pathogen contamination.

P3-22 DSC Quantification of *Escherichia coli* O157:H7 Transfer to Equipment during Commercial Production of Fresh-cut Leafy Greens

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Introduction: Recent *Escherichia coli* O157:H7 outbreaks associated with fresh-cut leafy greens have been traditionally traced to field contamination. However, considerable post-harvest cross-contamination can also occur during shredding, conveying, fluming and dewatering, with a current emphasis on recontamination from wash water.

Purpose: This study quantified *E. coli* O157:H7 transfer from dip-inoculated leafy greens to equipment surfaces during simulated commercial processing.

Methods: Three to five retail batches (22.7 kg) of baby spinach, iceberg lettuce and romaine lettuce were dip-inoculated to contain a 4-strain cocktail of avirulent, gfp-labeled, ampicillin-resistant *E. coli* O157:H7 at 10^6, 10^4 and 10^2 CFU/g, drained for one hour and processed using a commercial shredder (model TRS 2500 Urschel TranSlicer), step conveyor, 3.3-m flume tank equipped with two overhead spray jets, shaker table and centrifugal dryer (22.7 kg capacity). During processing, product (25 g) and water (40 ml) samples were collected from the step conveyer, flume tank, shaker table and/or dewatering centrifuge. After processing, 100 cm^2 product contact surface samples (n = 50) were collected from the shredder, conveyer, flume tank, shaker table and centrifuge, using Kimwipes®. Sample homogenates in phosphate or neutralizing buffer were plated on trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin with or without prior 0.45 µm membrane filtration to quantify gfp-labeled *E. coli* O157:H7 under UV light.

Results: During leafy green processing, 83 to97% of the *E. coli* O157:H7 inoculum was transferred to the wash water. After processing, *E. coli* O157:H7 populations were highest on the shredder (5.12 log CFU/g) and conveyor (5.26 log CFU/g) ($P < 0.05$), followed by the flume tank (3.85 log CFU/g) and shaker table (3.71 log CFU/g), with 30% of the remaining product inoculum lost during centrifugal drying.

Significance: This is the first report to quantitatively define both the role and importance of *E. coli* O157:H7 transfer from product to equipment surfaces during production of fresh-cut leafy greens. A dimensionless predictive model developed using a multi-modal process involving six transfer scenarios (pairwise transfers between product, water, and equipment) will be parameterized as additional data are generated. These findings will ultimately be invaluable in refining current microbial risk assessments being developed for fresh-cut produce.

P3-23 Use of the Systems Approach to Determine the Fate of *Escherichia coli* O157:H7 on Fresh and Fresh-cut Iceberg Lettuce and Spinach

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Introduction: Ideally when evaluating the fate of a pathogen on food the entire processing and handling practice would be studied as a system. Previous studies on contaminated produce have typically focused only on portions of the system. The systems approach allows individual components to be studied in order to see interactions rather than isolated situations.

Purpose: The systems approach was used to determine the fate of *E. coli* O157:H7 in the presence of normal background microorganisms on iceberg lettuce and spinach under conditions that mimic actual practices between production and retail sale.

Methods: Lettuce and spinach inoculated with *E. coli* O157:H7 were processed and handled in ways that might occur in commercial situations (including variations in holding times before and after product cooling, transportation conditions and temperatures, wash treatments, and product storage temperatures and times). Aerobic mesophilic and psychrotrophic bacteria, coliforms, yeasts and molds, lactic acid bacteria and E. coli O157:H7 populations were enumerated after each processing and handling step. Factorial ANOVA was used to predict the response variable with a combination of independent categorical variables and to test hypotheses about the means.

Results: Field temperature, time before cooling, and wash treatment significantly affected *E. coli* O157:H7 populations on both products. On greens packaged and stored at 4°C, *E. coli* O157:H7 contamination was detected, although populations decreased in many cases by at least 1.5 logs.

Significance: Factors in the system significantly affecting *E. coli* O157:H7 numbers from the time iceberg lettuce or baby spinach were harvested to the time products were put into retail bags were field temperature, time before cooling, and wash treatment. Time after cooling until lettuce was bagged was significant. However, for spinach this step was insignificant. *E. coli* O157:H7 contamination level on lettuce was not significantly different after vacuum cooling compared to before cooling.

P3-24 DSC Reduction of *Escherichia coli* O157:H7 in Fresh Spinach Using Chlorine and Lactic Acid Bacteria as a Multi-hurdle Intervention

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Introduction: Escherichia coli O157:H7 has increasingly become a problem in the fresh spinach and produce industry. Lactic acid bacteria (LAB) have proven effective as an intervention in raw and cooked meats and should be evaluated as a potential intervention in the production of fresh spinach.

Purpose: To determine if the post-harvest application of LAB and chlorine, alone or in combination as a multi-hurdle approach, can effectively control *Escherichia coli* O157:H7 throughout the shelf life of fresh spinach.

Methods: The ability of LAB to control *E. coli* O157:H7 populations, alone and in combination with the industry standard chlorine rinse, was determined in a 12 day shelf-life study at 7°C. The multi-hurdle intervention was evaluated in comparison to water, LAB and chlorine rinses. Results from all treatments were compared to an inoculated control. Lactic acid bacteria (Bovamine® Meat Cultures) were applied as a post-harvest rinse at a target concentration of 2.0×10^8 CFU/ml, while chlorine was utilized at the 200 ppm level. Spinach samples were held in a retail display cooler and tested for *Escherichia coli* O157:H7 on days 0, 1, 3, 6, 9 and 12, using the Neo-Grid Filtration system and CHROMagar. Survivability of LAB cultures was also determined.

Results: Significant reductions in *E. coli* O157:H7 populations were achieved by the water ($P = 0.0008$), LAB ($P < 0.0001$), chlorine ($P < 0.0001$) and multi-hurdle treatments ($P < 0.0001$) in comparison to control populations. However, the multi-hurdle treatment produced the greatest overall reductions, with a 1.91 log cycle reduction. This reduction was significantly better than water ($P < 0.0001$), LAB ($P = 0.0025$) and chlorine ($P < 0.0001$) alone, indicating that the application of chlorine and LAB is most effective as a combination treatment.

Significance: The standard chlorine wash utilized in the production of fresh spinach may be more effective at controlling *E. coli* O157:H7 if implemented as a multi-hurdle intervention in combination with LAB.

P3-25 Changes in Residual Chlorine Products Generated on Fresh-cut Lettuce after Chlorine Treatment

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Introduction: Because of the recent rise in fresh fruit and vegetable consumption, food poisoning caused by microbes is being observed more frequently. The most commonly used chemical stanitizer for fresh-cut food today is sodium hypochlorite at a concentration of 100 to 200 mg/l. However, sodium hypochlorite reacts to create residual products during the chlorine water processing, which may create toxicity problems.

Purpose: This study analyzes the residual chlorine content and trihalomethanes (THMs) of fresh-cut lettuce at varying concentrations of sodium hypochlorite, dipping times, numbers of washes, and temperatures of wash water, to verify the level of toxicity.

Methods: The chlorine residue in samples processed with chlorinated water was measured as follows. The sample was dipped for five minutes in ten-fold distilled water to create a sample solution. Then, by use of the method to analyze the quality of potable water, the sample solution was analyzed by the DPD method to measure the residual chlorine content, and by volatile organic compound-headspace/GC-ES 05601.3 to analyze THMs.

Results: When the concentration of sodium hypochlorite was under 200 mg/l, the residual amount of free chlorine was 1.0 to 7.0 mg/l. Although the rise in the residual amount was dependent on the concentration, the difference was insignificant. However, when the concentration of sodium hypochlorite exceeded 300 mg/l, the residual amount dramatically increased to 30.0 to 45.0 mg/l. The amount of free chlorine changed little with changes in dipping time. In contrast, the overall residual chlorine content rose slightly as the dipping time increased, after which it was sustained at a certain level. When the sample was washed once, the overall residual chlorine content stood at 16.0 mg/l. Residual chlorine was not detected when the sample was washed more than twice. At wash water temperatures of 5 to 20°C, the overall residual chlorine content was 22 to 28 mg/l. When the temperature was raised to 30 to 50°C, the content dropped to 5 to 12 mg/l, indicating that the residual chlorine content declined as the wash water temperature increased. The analysis for THMs under all conditions confirmed that there were no THMs remaining in fresh-cut lettuce.

Significance: The optimum washing conditions for lettuce in fresh-cut food consist of dipping the lettuce in chlorine stanitizer at a concentration of 100 to 200 mg/l and a temperature of 20°C for one minute, and then washing it twice with clean water, each time for 30 s.

P3-26 Modeling the Growth of *Salmonella* on Cut Tomatoes

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Introduction: Outbreaks of salmonellosis associated with fresh tomatoes continue to be major food safety concern. When whole tomatoes are cut, any *Salmonella* present can be transferred from the surface to the inside of the fruit. Despite their low pH, cut tomatoes provide a favorable environment for *Salmonella* to grow when temperature abused.

Purpose: Few reports quantify the growth rate of *Salmonella* on cut tomatoes, and only at a limited number of incubation temperatures. While modeling software (the pathogen modeling program — PMP and ComBase Predictor - CBP) can be used to predict *Salmonella* growth, no models have been validated for cut tomatoes. Our purpose was to build a mathematical model to describe the growth of *Salmonella* on cut tomatoes and to compare that model with other published *Salmonella* growth models.

Methods: Whole red round tomatoes were dip inoculated with a cocktail of four *Salmonella* strains obtained from the Centers for Disease Control and Prevention (CDC). These strains are all human isolates associated with past tomato outbreaks. Inoculated tomatoes were dried overnight, cut with a sterile knife, placed in a sterile plastic bag, incubated at one of 10 different temperatures and plated on XLT4 agar for enumeration. Growth rates were calculated using DMFit.

Results: A plot of the square root (SR) of growth rate (GR) was linear with temperature (T), such that SR(GR) = 0.026T - 0.1065, (R^2 = 0.9398). The growth rate was slightly faster than that predicted by CBP, but slower than that predicted by PMP. CBP would allow predictions at the pH of cut tomatoes (4.3), while the lowest pH available for *Salmonella* growth in the PMP was 5.6.

Significance: The *Salmonella* in the cut tomato model created here provides a useful tool for estimating the risk posed by different abuse temperatures. The model also provides validation of CBP as a model for *Salmonella* growth in cut tomatoes.

P3-27 High Pressure Processing to Reduce *Salmonella enterica* from Broth and Diced Tomatoes

DSC

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Introduction: Fresh and fresh-cut tomatoes have been associated with numerous outbreaks of salmonellosis in recent years. While the exact routes of contamination are unknown, high pressure processing (HPP) is being evaluated as a post harvest treatment to eliminate *Salmonella*.

Purpose: The objectives of the study were to determine the effect of HPP at three pressures on reduction of *S. enterica* serovars Newport, Javiana, Braenderup and Anatum (clinical isolates from tomato outbreaks) in Tryptic Soy Broth and to determine the effect of HPP on reduction of the most pressure resistant *S. enterica* serovar from fresh diced tomatoes.

Methods: Separate 5 ml portions of broth containing 8 log CFU/ml of each of the four serovars (made nalidixic acid resistant) were packaged in sterile stomacher bags and subjected to three different pressures (350, 450, and 550 MPa) for 120s. Diced tomatoes (150g) were inoculated with the most pressure resistant serovar as determined by the preceding work, to obtain 6 log CFU/g throughout the sample, and subjected to the same pressure treatments as described above. Treated broth and tomato samples (homogenized for 1 min) were surface plated onto Tryptic Soy Agar supplemented with nalidixic acid and incubated at 35°C for 48 h.

Results: The most pressure resistant serovar of *Salmonella* evaluated was Braenderup. Subjecting the broth culture to 350, 450 and 550 MPa resulted in a 4.90, 6.10 and 7.45 log reduction in *S.* Braenderup, respectively. No significant reductions were seen in *S.* Braenderup concentrations in diced tomatoes when processed at 350 MPa. Significant reductions ($P < 0.05$) were seen at 450 (1.63 log CFU/g) and 550 MPa (3.65 log CFU/g). There were no differences in visual appearance between fresh diced and HPP diced tomatoes.

Significance: HPP may be an effective post harvest strategy to reduce low levels of *Salmonella* contamination in diced tomatoes.

P3-28 Factors Affecting Infiltration, Survival, and Growth of *Salmonella* on Inshell Pecans and Pecan Nutmeats

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Introduction: Outbreaks of foodborne infections have been associated with consumption of tree nuts. Two outbreaks of salmonellosis associated with almonds and one outbreak of *Escherichia coli* O157:H7 infection associated with cashew nuts have been documented. Pecan handling and processing practices have potential for enhancing or compromising safety. However, factors that may affect infiltration, survival, and growth of foodborne pathogens on pecans have been given only meager research attention.

Purpose: The purpose of this study was to determine (1) the effect of temperature on infiltration of *Salmonella* into inshell pecans; (2) survival characteristics of the pathogen on and in inshell pecans and pecan nutmeats as affected by temperature during long-term storage; and (3) survival and growth characteristics of *Salmonella* on high-a_w nutmeats.

Methods: Infiltration of *Salmonella* into inshell pecans as affected by temperature differential (nuts at -20, 4, 21, and 37°C; suspension at 21°C) was studied. Survival of the pathogen on and in dry inshell pecans, pecan halves, and pecan pieces during storage at -20, 4, 21, and 37°C was monitored. Survival and growth characteristics on high-a_w (0.96 to 0.99) nutmeats held at 4, 21, 30, and 37°C were determined.

Results: The rate of infiltration of water into inshell pecans was greater when pecans were at a temperature higher than that of the water. *Salmonella* was isolated from nutmeats of inshell pecans that had been immersed in a cell suspension, dried, and stored at 4°C for 4 weeks, indicating that the pathogen can infiltrate the nut, reach the edible portion, and survive. Populations of *Salmonella* on and in inshell pecans (nutmeat a_w, 0.43 to 0.51; moisture, 3.2 to 3.6%) and nutmeats (a_w, 0.53 to 0.63; moisture, 3.7 to 3.8%) stored for up to 36 weeks at -20 and 4°C decreased slightly; significant decreases occurred at 21 and 37°C but viable cells were detected. The pathogen grew at 21, 30, and 37°C but not at 4°C on high-a_w (0.96 to 0.99) pecan halves, pieces, and granules.

Significance: The ability of *Salmonella* to infiltrate pecans and survive for long periods of time, coupled with its ability to grow on high-a_w nutmeats, emphasizes the importance of applying process treatments that will inactivate the pathogen.

Poster

P3-29 Behavior of *Salmonella* Inoculated onto Walnut Hulls before and during Harvest and Hulling

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Introduction: Salmonellosis has been associated with consumption of many dried foods, including almonds and peanut butter. The behavior of *Salmonella* during postharvest handling of almonds has been relatively well studied, but little is known about this organism and other tree nuts.

Purpose: To determine the behavior of *Salmonella* on walnut hulls before and during harvest and hulling.

Methods: Immature and mature walnuts were picked from two trees growing in a university orchard, and the hull was removed from the shell. Well water, hulling water, and hulls were also collected from a local walnut huller. Rifampicin-resistant *Salmonella* Enteritidis PT30 was inoculated onto hulls, and water and hull/water mixtures at 2 to 9 log CFU/g or ml. Samples were stored at ambient temperature at high or low relative humidity (RH) for up to 2 weeks. *Salmonella* was recovered from intact hulls by stomaching in 0.1% peptone and all samples were plated onto bismuth sulfite agar containing rifampicin.

Results: When inoculated onto hulls at 3, 5, and 9 log CFU/g *Salmonella* levels increased within 2 days to 6 log CFU/g, and were stable or decreased slightly, when stored at higher RH (> 40%). In contrast, when stored at low RH (~ 20 %) levels of *Salmonella* decreased to < 0.3 log CFU/g within 24 h. When hulls were blended in water and inoculated immediately, *Salmonella* declined by 2 log CFU/ml to 1 CFU/ml within 24 h. A delay in inoculation of 24 h reduced that decline to 0.5 log CFU/ml. Similarly, levels of *Salmonella* decreased to 1 CFU/ml in well water that was exposed to hulls for 30 or 60 min. In collected hull waste water, populations remained stable within a 24 h period.

Significance: The ability of *Salmonella* to survive or grow in walnut hulls and hull waste water was dependent upon RH and unidentified hull components. Regularly removing hull debris in walnut hulling operations may help reduce potential reservoirs for *Salmonella* in these facilities.

P3-30 Ozone Inactivation of Norovirus Surrogates on Fresh Produce

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Introduction: Preharvest contamination of produce by foodborne viruses can occur through feces, soil, irrigation water, animals and human handling, and this produce has the potential to be distributed throughout the entire country. Greater than 50% of norovirus outbreaks are associated with the consumption of salads, sandwiches and fresh produce. Chlorine use on fresh produce has shown to be minimally effective for viral inactivation; however, ozone is effective for a wide range of viruses.

Purpose: Ozone inactivation of bacteria on fresh produce, including apples, strawberries and lettuce, has been demonstrated to be effective; however, there is a lack of research on viral inactivation on produce by ozone. This study compares the inactivation of human norovirus surrogates (feline calicivirus and mouse norovirus) on green onions and lettuce by treatment with ozone.

Methods: Cut pieces of green onions and lettuce (5 g) were inoculated with FCV (10^7 TCID50/mL) or MNV (10^6 PFU/mL). Green onions and lettuce were placed in 45 mL of sterile water (pH 7.0) and treated with ozone (0.9 g/h) for 1, 5 and 10 min time intervals. After ozone treatment, sodium thiosulfate (5%) was added to quench residual ozone and samples were stomached before determination of infectivity by TCID50 analysis or plaque assay.

Results: MNV inoculated produce showed greater inactivation than FCV, as shown by 4.1 ± 0.2 log PFU/mL MNV inactivation after a 10 min treatment of green onions compared to a less than 1.8 ± 0.16 log TCID50/mL FCV inactivation. Inactivation of FCV did not increase with treatment time, as inactivations of 1.92 ± 0.59 and 1.78 ± 0.16 log TCID50/mL were observed for 1 and 10 min, respectively.

Significance: These results indicate that ozone is an effective method to reduce viral contamination on the surface of fresh produce. Interestingly, norovirus surrogates displayed varying sensitivity to ozone.

P3-31 Parasite Resistance to Peroxiacetic and Citric Acid-based Disinfectants

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Introduction: Contaminated fresh produce are being implicated in foodborne outbreaks. As more fresh produce are being imported into the US, food and waterborne parasites are more likely to be introduced into the US food supply. An alternative used by the fresh produce industry to control and eliminate bacterial pathogens in these commodities include the use of sanitizers.

Purpose: The effect of peroxyacetic and citric acid-based sanitizers on the infectivity of enteric pathogens was examined in the present study.

Methods: Encephalitozoon intestinalis, *Cryptosporidium parvum*, and *Cyclospora cayetanensis* were used in the present study. *E. intestinalis* and *C. parvum* viability were examined in tissue cultureRK-13 and Caco-2 cell lines respectively. Feline calicivirus F9 (viability tested in CRFK cell lined) and two isolates of nalidixic resistant *Escherichia coli* O157:H7 were also examined.

Results: Cryptosporidium parvum Iowa isolate and *E. intestinalis* vialibility, when tested in vitro, were not affected by the peroxiacetic based sanitizers at 80 ug/ml at 5 min treatment. At 15 min a 2 log reduction was observed with microsporidia but not with Cryptosporidium. Feline calicivirus reduction of > 1.5 log was observed at 5 min treatment and more than 2 logs when treated for 15 min. A reduction of 2 to 4 log reduction was observed when *E. coli* O157:H7 was treated for 5 and 15 min. Calicivirus reduction of > 4 logs was obtained by treatment with the citric acid based sanitizer (10 mg/ml)whereas no reduction was observed for *Cryptosporidium* or microsporidia.

Significance: Parasites are more resistant than *E. coli* O157:H7 to these two disinfectants tested. Identification of effective sanitizers against parasites still need to be identified.

P3-32 Effects of Compost Tea and Compost Socks on Microbiological and Harvest Quality of Strawberry Fruits

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Introduction: Compost tea (CT) is considered by some organic and conventional growers as a cost-effective, biologically-based control for several foliar and root diseases when used as a spray or soil drench. CT has been shown to enhance strawberry fruit yields as well as reduce disease severity of *Botrytis cinerea* (grey mold), a fungal fruit rot. CT may be a source of foodborne pathogens if the ingredients or the brewing processes are not properly controlled.

Purpose: This study determined the effects of two compost tea treatments on *E. coli* contamination, yield, and plant disease of strawberry fruits grown in black root-rot infested soil and compost socks.

Methods: Two nutrient-supplemented aerated compost tea treatments (CT and CT amended with three yeast isolates known as biocontrols for fungal rot of fruit) and a water spray control were applied in a split-split plot treatment design to four strawberry cultivars (Sparkle, Chandler, Northeastern, Allstar) grown in Maryland either in sandy loam soil with a history of black root rot or poultry litter compost-filled socks. The CT used in this study was naturally contaminated with 2.73 CFU/ml commensal *E. coli* and was applied at a rate of 250 ml per linear meter of plant bed (approximately 40 ml per plant). Strawberry yields, percentage of diseased fruits and microbiological quality (total heterotrophs, gram negatives, total yeast, enterococci, coliforms and *E. coli*) were determined for seven fruit harvest events in June 2005.

Results: Compost tea treatments did not have significant effects on either harvest yield or percentage of diseased fruits when compared to water spray controls. Although each plant was thoroughly sprayed with CT, resulting in deposition of ~100 CFU *E. coli* per plant, *E. coli* was not detected on any fruits that matured four days post CT application. Three cultivars (Allstar, Chandler and Northeastern) produced greater yields when grown in compost socks (272.9, 146, 124.6 g/lin-m, respectively) than in soil (148.7, 88.1, 93.7 g/lin-m, respectively). Furthermore, cultivars grown in compost tended to have fewer (2 to 10%) diseased fruits than those grown in unamended soil beds, although these results were not significantly different ($P > 0.05$).

Significance: This study provides data to support organic methods to enhance strawberry crop yield and reduce disease incidence. Survival data for *E. coli* shows that low-dose foliar spray application of CT containing very low concentrations of *E. coli* at least four days with high solar radiation before harvest leads to complete loss in viability of *E. coli*.

P3-33 Efficacy of Consumer-available Antimicrobials for In-home Surface Disinfection of Produce

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Introduction: Produce safety remains a concern for the food industry and consumers desiring a nutritious and healthful diet. The consumer represents the last line of defense in the protection of food safety. However, relatively little information exists on the efficacy of simple, home-based methods for the surface disinfection of fresh produce.

Purpose: The purpose of this study was to determine the efficacy of consumer-available antimicrobials in decontaminating the surface of bell peppers.

Methods: Unwaxed bell peppers were inoculated with a bacterial cocktail containing rifampicin-resistant *Salmonella enterica* serovars Agona, Michigan, Poona and Typhimurium to 5.0 log CFU/30 cm^2. Peppers were dipped for 10 s in sterile distilled water, 2.5% acetic acid, 3% hydrogen peroxide, or 70% ethanol. A control group consisted of inoculated peppers not subjected to any treatment. Surviving salmonellae on all treatment groups were enumerated by spread plating on Tryptic Soy Agar containing 100 mg/L rifampicin. Plates were incubated aerobically at 35°C for 48 h prior to pathogen enumeration. These experiments were completed in triplicate.

Results: Dipping in sterile water produced no significant reduction in the population of inculated *Salmonella* (0.9 log CFU/30 cm^2) ($P < 0.05$). In contrast, counts on peppers exposed to 2.5% acetic acid, 3% hydrogen peroxide and 70% ethanol were 2.9, 2.5, and >4.0 log cycles, respectively, lower than counts on the control group.

Significance: Experimental results suggest that the disinfection of bell peppers in the home using antimicrobials easily accessible to consumers can improve the safety of bell peppers prior to consumption.

P3-34 DSC Washing Effect of Sodium Hypochlorite with 5% Acetic Acid on the Vegetative Cells and Spore of Pathogenic Microorganisms and Sensory Quality of Fresh Produce

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Introduction: Washing fresh produce with sanitizer is one of the most important intervention steps during the processing of fresh produce. However, equal effectiveness of different sanitizers is questionable because of differences of the types of pathogen, sanitizer, produce, physiological state, etc.

Purpose: The objectives of this study were to evaluate the washing effects of sodium hypochlorite (Cl) and Cl with 5% acetic acid on the reduction of pathogens inoculated onto fresh produces and to measure the effect of washing on the sensory quality of fresh produce.

Methods: S. Typhimurium, *L. monocytogenes* and *B. cereus* were inoculated into 10 g of cherry tomato, cucumber and carrot. They were washed for 5 or 10 min in 100 or 200 ppm Cl or Cl with 5% acetic acid. The inoculated samples were dipped into 100 ml sanitizer solution and shaken at 100 rpm for 5 min, and then stored at 4 and 10°C for 10 days for sensory evaluation and microbiological test.

Results: The shaking method was significantly ($P < 0.05$) more effective than the dipping method, regardless of the type of produce, pathogen and concentration of sanitizer. Vegetative cells were reduced significantly ($P < 0.05$) than spore in the case of *B. cereus*. *S.* Typhimurium and *L. monocytogenes*. Cells attached on the produces for 24 h were much more resistant

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to the sanitizers. The 100 ppm Cl with 5% acetic acid was more effective in microbial reduction during 10 days of storage, however, the lowest sensory scores of color, flavor, texture and overall acceptability were observed in sample washed with 100 ppm Cl with 5% acetic acid, regardless of the type of produce after 10 days.

Significance: Washing with 100 ppm Cl with 5% acetic acid can be recommended as an effective means of enhancing the safety of fresh produce. However, it is inappropriate for produce kept in refrigeration for more than 5 days.

P3-35 Reduction of Pathogenic Bacteria on Avocados by Washing Treatments

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Introduction: The potential for superficial contamination of avocados by pathogenic bacteria has recently been established. In fact, most of the plants that process guacamole and minimally processed avocado in Mexico are encouraged to apply a surface decontamination treatment prior to processing. Although various chemical sanitizers are commercially available, validation studies to prove their efficacy are lacking.

Purpose: The purpose of this study was to compare the efficacy of different washing procedures at reducing *Listeria monocytogenes*, *Salmonella* and *Escherichia coli* O157:H7 on the surface of avocados.

Methods: Fresh avocados (Hass) were inoculated with a bacterial cocktail containing rifampicin resistant (Rif +) *L. monocytogenes* or Rif + *Salmonella* and *E. coli* O157:H7, and then subjected to washing with: tap water, hot water (70°C), lactic acid (2%, 55°C), peracetic acid (80 ppm), chlorine (200 ppm) or a commercial formulation based on natural substances. After treatment, reductions in population were determined by plate counting and physical determinations, as weight, texture, firmness and skin color, were performed.

Results: Hot water and lactic acid treatments produced the greatest reductions of the 3 pathogenic bacteria (2.6 – 3.4 log/avocado). The reductions obtained by peracetic acid, chlorine or the commercial formulation (< 2 log/avocado) were not different from the reductions obtained by a sole tap water wash. None of the treatments affected weight, texture, firmness or skin color of the fruits.

Significance: Lactic acid and hot water treatments reduced *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 by ~3 log cycles on Hass avocados without affecting the fruit quality. Reduction with hot water may have an advantage over treatment with lactic acid by leaving no chemical residues on the fruit.

P3-36 The Effect of Gaseous Ozone on the Shelf Life and Sensory Properties of Fresh Lychees

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Introduction: The post-harvest spoilage of fresh produce is of global economic importance. Spoilage is often controlled by fungicides such as imazalil and thiabendazole, but microbial resistance and human health risks have led to an increased demand for alternative technologies. Exposure to gaseous ozone on post harvest decay of different commodities during storage has been found to inhibit aerial mycelia and prevent sporulation of mold spores.

Purpose: This study investigated the effect of gaseous ozone on the shelf life of fresh lychees (*Litchi chinensis* Sonn.). Microbiological and sensory quality parameters (appearance and texture) were investigated.

Methods: Seventy crates, each containing 2 kg of lychees, were stacked 10 crates high and treated with gaseous ozone for 2 h at a concentration of approximately 24 ppm in a Biosafety level 2 bioaerosol test chamber. The fruit was then packed into trays and over-wrapped to mimic the conditions prevalent during subsequent distribution and retail storage. Control samples were also prepared, which were not subjected to ozone treatment, but wrapped and stored in an identical manner. The level of fungal contamination of the pedicarp was analyzed using surface plate count. The color of the pedicarp was analyzed visually and the texture of the pedicarp and fruit (penetration and withdrawal forces) was measured using a Penetrometer.

Results: There was a significant difference ($P \leq 0.05$) between mold counts on ozonated and non-ozonated (control) lychees. A visual assessment after 15 days showed this marked difference. There was a significant difference ($P \leq 0.05$) between the top crates compared with crates taken from middle and bottom portion of the stack, with those at the top showing lower mold counts. There was, however, no significant difference between the middle and bottom crates. There were no significant differences in color (pedicarp only) or texture (pedicarp and flesh) of ozonated and control fruit. The effect of ozone on the texture showed a softening of the pedicarp but no significant differences in softness or 'stickiness' of the fruit.

Significance: These results suggest that the application of gaseous ozone to fresh lychees can increase shelf life by 4 days. This is dependent on the position of the crates during treatment, as the ozone must be allowed to circulate freely around the produce. Gaseous ozone treatment for the prevention of post harvest decay and the extension of the shelf life of produce has the potential for application within the food industry.

P3-37 Fate of *Salmonella* spp. on Fresh and Frozen Cut Mangoes
DSC

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Introduction: Imported raw mangoes have been associated with three US outbreaks of *Salmonella*.

Purpose: Our objective was to evaluate the fate of *Salmonella* on fresh (23, 12 and 4°C) and frozen (-20°C) cut mangoes.

Methods: Cut mangoes were spot inoculated with a five strain cocktail of *Salmonella* at 5 log CFU/g (-20 ± 2°C) or 1, 3 and 5 log CFU/g (all other temperatures). Samples were enumerated following stomaching on selective and non-selective media at days 0, 1, 3, 5 and 7 (23 ± 2°C); 0, 1, 3, 5, 7, 10, 14, 21 and 28 (12 ± 2 and 4 ± 2°C); and 0, 7, 14, 21, 28, 60, 90 and 120 (-20 ± 2°C). Population levels in log CFU/g were calculated.

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Results: Mangoes had visually spoiled by day 3 or 5 at 23 ± 2 or 12 ± 2°C, respectively. At 23 ± 2°C, population increases on day 1 of 2, 3 and 3 log CFU/g were seen for initial inoculum concentrations of 5, 3, and 1 log CFU/g respectively. An additional 3 log CFU/g increase was observed on day 3 for mangoes initially inoculated at 1 log CFU/g. At 12 ± 2°C, a population increase on day 1 of 2 log CFU/g was seen for 5 log CFU/g inoculations. Following no change on day 1, a 3 log CFU/g increase was observed on day 3 for 3 log CFU/g inoculations. With inoculation of 1 log CFU/g, populations remained stable for 28 days. At 4 ± 2°C, populations stabilized at ca. 3.5, 2 and 0.5 log CFU/g for the duration of the experiment for initial inoculum concentrations of 5, 3, and 1 log CFU/g, respectively. At -20 ± 2°C, populations declined ca. 2 log CFU/g in the first 7 days, before stabilizing at 3 log CFU/g for 120 days.

Significance: Salmonella has the potential to grow on temperature-abused fresh cut mangoes, and to survive for the shelf life of both fresh and frozen cut mangoes.

P3-38 Growth and Survival of *Salmonella* Enteritidis in Mango Pulp

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Introduction: Understanding the growth and survival of *Salmonella* Enteritidis in mango pulp (*Mangifera indica* Linn; var. Palmer) at different times and temperatures will be helpful in developing effective interventions for the control of this pathogen in this fruit.

Purpose: To determine the growth and survival of *Salmonella* Enteritidis in mango pulp at different temperatures and incubation times.

Methods: Fruits without any defects (peel ruptures, bruised areas) were used in the experiment. Pulp was aseptically removed and homogenized in a previously sterilized blender. Mango portions were inoculated with saline suspensions of the test organisms, yielding final populations of approximately 700 CFU/g. The inoculated pulps were incubated at four different temperatures (-20°C, >4°C, 10°C and 25°C) and incubation times. At each sampling time, 1 ml of fruit pulp was collected, serially diluted in peptone water and pour plated into TSA (45°C). The plates were incubated at 35°C for 24 h and bacteria were then counted, with results being expressed in CFU/g. Uninoculated pulp controls were also analyzed to ensure the absence of any background microflora before and after the incubation time.

Results: Generation times of 1 h and 26 h were obtained for *S.* Enteritidis at temperatures of 25°C and 10°C, respectively. Survival of *S.* Enteritidis was observed at 4°C and -20°C after 10 days and 5 months, respectively.

Significance: The study indicates that *S.* Enteritidis can grow well in homogenized mango pulp at 25°C and that a low temperature (10°C) retards but does not stop the growth of this bacterium. If previous contamination of mango pulp by *S.* Enteriditis occurs, the temperatures of 4°C and 10°C cannot be considered safe conditions for keeping this fruit.

P3-39 Microbial Quality of Fresh Hand-picked Ontario-grown Fruits

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Introduction: Fresh fruits are often sold through U-pick farms and in farmers' markets in Ontario during the harvest season, but there is a potential risk of bacterial contamination from the fields or from postharvest handling by consumers or workers.

Purpose: This study was to determine the microbiological quality of hand-picked fresh fruits produced in Ontario, including strawberry, cherry, peach and apple. The microbial analyses focused on *Escherichia coli*, *Listeria monocytogenes*, *Shigella* spp., fecal streptococci and total coliforms.

Methods: The fruit samples were picked either by workers (treatment) or researchers (control) (after washing hands with 70% ethanol) directly in the orchards or obtained from farmers' markets, and were kept on ice or in a refrigerator at 4°C before being processed. The methods recommended by Health Canada (Health Canada Compendium of Analytical Methods) were used for the analyses of the targeting microorganisms, respectively.

Results: Among the total 155 samples tested, *E. coli* was detected on two strawberry samples, at the level < log 3 CFU/g fruit, resulting in a prevalence of 1.3% (2/155) for all produce and 5.3% (2/38) for strawberry. The two samples were obtained from the farm gate and picked by workers. Neither *L. monocytogenes* nor *Shigella* spp. were detected in any of the tested samples. Fecal streptococci were detected in 5.3% (2 out of 38), 8.8% (3 out of 34), 8.3% (3 out of 36) and 6.4% (3 out of 47) of strawberry, cherry, peach and apple samples, respectively, ranging from 1.0 to 3.6 CFU/g. Coliforms were detected in 100, 56, 30 and 47% of strawberry, cherry, peach and apple samples, respectively, with values ranging from 0.1 to 3.8 CFU/g. There was generally no significant difference between the researcher (aseptically) picked and worker picked fruit samples.

Significance: The results provide needed quantitative data on the microbial load of fresh hand picked fruits that can be utilized to fill gaps in existing risk assessments.

P3-40 *Salmonella* Transfer Potential Associated with the Hand-peeling of Citrus

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Introduction: The waxy surface of citrus fruits provides conditions suitable for bacterial adherence. Citrus fruit are commonly hand peeled prior to eating; the potential for pathogen transfer from the peel to the edible portion of the fruit during this step.

Purpose: The objective of this study was to determine rates of transfer from surface-contaminated citrus fruit that occur as a result of hand peeling. Transfer rates were determined between the whole fruit and the peel, edible segments and gloved hands.

Methods: A virulent, rifampicin-resistant *Salmonella* Typhimurium was inoculated onto the stem, styler, or equator of three common fresh citrus varieties (Valencia oranges, Satsuma mandarins, and Marsh grapefruit), and allowed to dry for 24 h. Six volunteers, three men and three women, hand peeled the fruit and collected the peel, edible segments, and gloves into whirl-pak bags. Each sample was massaged for 30 s in DE neutralizing buffer and enumerated from Tryptic Soy Agar supplemented with rifampicin. The influence of gender and citrus variety on the percentage of bacteria transferred per recipient surface was determined using statistical analysis.

Results: No significant difference ($P \leq 0.05$) was found between men and women or location of inoculum on Marsh grapefruit or Valencia oranges. The peel portion of Satsuma mandarins showed significant differences in gender and inoculation site. Percent transfer to edible segments ranged from 27.9% to 0% in Valencia, 5% to 0.1% in grapefruit and 3.5% to 0% in mandarins. Transfer to gloved hands ranged from 10% to 0% in Valencia, 2.1% to 0.1% in grapefruit and 11.6% to 0% in mandarins. Pathogens transfer from the whole fruit to edible portions and hands regardless of the location of inoculation.

Significance: Pathogens present on the surface of fresh citrus have the potential to transfer to the edible portion during hand peeling.

P3-41 Distribution of *Listeria monocytogenes* in a Frozen Spinach Plant: Impact of Changes on Sanitation Procedures on Contamination Patterns

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Introduction: Eating raw produce and *Listeria monocytogenes* have been associated to illness outbreaks. This pathogen, a gram positive rod of wide distribution in the environment, is able to thrive in food production facilities. To establish effective control measures for *L. monocytogenes*, it is necessary to generate objective information based on a scientific approach.

Purpose: Our objective was to study the impact of sanitation procedures on the contamination profile of *L. monocytogenes* in a processing plant that produces precooked frozen spinach.

Methods: The facilities were visited seven times during a three-month period. Samples of inert surfaces, water, and spinach (raw, precooked and frozen) were collected to detect *L. monocytogenes* using both USDA and FDA methods. Isolated *L. monocytogenes* strains were subjected to pulsed-field gel electrophoresis using *Apa*I and *Asc*I.

Results: A total of 412 samples (259 spinach, 122 surfaces, and 31 water) were collected. In the first three studies, the global incidence of *L. monocytogenes* was 18.4% (+/n = 37/201). After the third study, data were analyzed and changes to sanitation procedures were introduced, including new methods for cleaning and sanitizing equipment, utensils, and floors. The incidence of the pathogen for studies four to seven was 1.4% (+/n = 3/211). Spinach residues remaining on equipment was the most contaminated type of sample for both sampling periods. *L. monocytogenes* strains were grouped into eight and six pulsotypes with *Apa*I and *Asc*I, respectively. One pulsotype was the most frequent and widely distributed in the processing plant (57.1%) even after the intervention steps were modified.

Significance: Data suggests that specific control strategies for each food facility may be indispensable to control *L. monocytogenes* in a plant environment and reduce potential cross-contamination.

P3-42 A Review of Gastrointestinal Outbreaks in Schools: Recommendations to Reduce Illness

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Introduction: Confined environments, such as schools, provide opportunities for direct and indirect transmission of disease agents when children come in close contact with each other or when children touch common surfaces. Food offered to students through a lunch program or the cafeteria can become another means of transmission through contamination from an infected food worker or improper handling of raw products, especially meats. Exposure to contaminated water and educational field trips to farms may provide other exposures.

Purpose: The purpose of this study was to review documented outbreaks of enteric illness in schools, published in the last ten years, to identify aetiology, mode of transmission, the number of children affected, and morbidity and mortality patterns.

Methods: Searches were completed in electronic databases, public health publications and Federal, state and provincial public health Web sites. Reference lists were hand-searched to validate the electronic search methodology. Reports identified through personal communications with public health officials were included.

Results: Etiology of the 121 outbreaks that met the inclusion criteria included: bacterial 50%, viral 40%, *Cryptosporidium* 7%, and multiple organisms 2%. Transmission routes recorded in 101 reports included: foodborne (45%), person-to-person (16%), waterborne (12%), and animal contact (11%). Actions to control the outbreak included: alerting public health authorities, health care providers, or the community to the outbreak (13%), treatment of cases and close contacts (12%), enhanced handwashing (11%) and increased vigilance during food preparation (8%). Recommendations were made retrospectively to prevent future outbreaks in 77 (64%) reports.

Significance: Training and certification of food handlers could prevent foodborne outbreaks in schools. Children should be supervised on farm visits, handwashing strictly enforced and food eaten in an area separated from the animals. Staff and students should have a positive and continuous communication with public health authorities, including educational sessions and immediate reporting of possible outbreaks.

P3-43 Recurrent *Salmonella* Anatum Outbreaks Linked to Pulled Pork Barbecue—TN, 2006 to 2008

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Introduction: Salmonella spp. are frequently associated with foodborne illness. The Centers for Disease Control and Prevention (CDC) estimates 1.4 million cases of salmonellosis occur in the US each year.

Purpose: The investigation objectives were to describe the outbreak, implement control measures, and prevent additional illnesses. We used CDC's Outbreak Management System (OMS) Version 1.2 in the investigation of a multi-jurisdictional *Salmonella* Anatum outbreak in Tennessee. This was the first documented use of OMS in a foodborne disease outbreak.

Methods: Ill persons were initially identified by area physicians and by routine laboratory reporting. Additional cases were identified via active surveillance. All patients were interviewed using standardized FoodNet and *Salmonella* questionnaires. Stool and food specimens were tested for enteric pathogens. OMS and NetDraw were used for data management and visualization.

Results: In the 2006 outbreak, fifty-five ill persons were identified, including 11 laboratory-confirmed cases and 44 probable cases which were epidemiologically linked to confirmed cases. In 2008, fifty (96%) of 52 persons interviewed were ill, including 13 laboratory-confirmed cases and 37 probable cases which were epidemiologically linked. All laboratory-confirmed cases were positive for *Salmonella* Anatum, *Xba*I pattern JAGX01.0001, as were pulled pork barbeque specimens collected from ill persons in 2008. In 2008, fifty (96%) out of 52 persons who consumed pulled pork barbecue meat became ill.

Significance: In two separate outbreaks, a total of 105 confirmed and probable cases of salmonellosis were linked to contaminated pulled pork barbeque from a single restaurant/catering establishment. *Salmonella* is not considered an adulterant of meats. Risks with pulled pork barbeque were reviewed with the restaurant including appropriate cooking temperatures, handling, and ways to mitigate cross contamination during preparation. OMS was a valuable asset for tracking specimens, identifying relationships between cases, and identifying relationships between cases and exposure events. Social network analysis provided a visual depiction of epidemiological links between cases, potential exposure events and laboratory specimens. Use of OMS and social network analysis should be considered in future investigations.

P3-44 Enteric Disease Outbreaks Associated with Consumption of Fresh Melons — United States, 1998 to 2007

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Introduction: Fresh produce is an important part of a healthy diet and consumption has increased in recent years. In particular, melons that are consumed raw have been associated with several large outbreaks of enteric infections in the US.

Purpose: A better understanding of the epidemiology of melon-associated outbreaks is necessary to guide public health and regulatory interventions.

Methods: We reviewed 1998 to 2007 data from the Foodborne-Disease Outbreak (FBDO) surveillance system in which food items implicated in outbreak investigations were classified into 17 food commodities: fish, crustaceans, mollusks, dairy, eggs, beef, game, pork, poultry, grains-beans, oils-sugars, fruits-nuts, fungi, leafy vegetables, root vegetables, sprouts, and vegetables from a vine or stalk. A melon-associated FBDO is defined as two or more illnesses due to the consumption of a single food vehicle identified as a melon (cantaloupe, honeydew, or watermelon) or consumption of at least one fruit item identified as a melon in a multiple ingredient food vehicle in which all the ingredients belonged to the fruits-nuts commodity (fruit salad).

Results: Of the 11,976 single etiology FBDOs reported during 1998–2007, 6,989 (58%) implicated a food item, 2,993 (25%) implicated food items that could be classified to a single commodity, 162 were attributed to the fruits-nuts commodity; of these, 61 (38%) outbreaks classified as melon-associated caused 2,640 illnesses, 127 hospitalizations, and 6 deaths; the median size of melon-associated outbreaks (26.5 illnesses) was more than three times the median size of non-melon associated outbreaks (7). Among melon-associated FBDOs with a confirmed etiology, norovirus was responsible for 19 (46%) outbreaks and 725 (34%) illnesses, followed by *Salmonella* with 15 (37%) outbreaks and 515 (24%) illnesses. Six (40%) *Salmonella* outbreaks were due to cantaloupe (3 outbreaks), honeydew melon (1), both cantaloupe and honeydew (1), and fruit salad (1) and all had multistate exposures indicating that contamination likely occurred before preparation, whereas the largest melon-associated outbreak reported was due to watermelon cross-contaminated with *Escherichia coli* serotype O157:H7 at a restaurant.

Significance: Understanding the epidemiology of melon-associated outbreaks is critical in guiding control efforts. Contaminated melons cause both multistate and individual restaurant-associated outbreaks; efforts by local, state, and federal agencies to control melon-associated outbreaks should span from the point of harvest to the point of preparation; attention should be placed on decreasing contamination of cantaloupes and honeydew melons with *Salmonella*.

P3-45 Epidemiology of Multistate Foodborne Outbreaks, United States: 1998 to 2007

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Introduction: Contaminated products that are distributed on a large scale can cause foodborne disease outbreaks (FBDOs) involving multiple states. Due to geographic dispersion, these outbreaks can appear to be unassociated clusters of sporadic cases; however, due to improvements in surveillance, more multistate outbreaks are being detected.

Purpose: The purpose of this study is to describe the epidemiology of multistate foodborne outbreaks.

Methods: We reviewed data from CDC's electronic Foodborne Outbreak Reporting System (eFORS) for 1998 to 2007. Foods implicated in outbreak investigations were classified into 17 food commodities: fish, crustaceans, mollusks, dairy,

eggs, beef, game, pork, poultry, grains-beans, oils-sugars, fruits-nuts, fungi, leafy vegetables, root vegetables, sprouts, and vegetables from a vine or stalk. A multistate FBDO was defined as the occurrence of two or more illnesses resulting from a common food exposure, in which exposure occurred in multiple states.

Results: Among 12,398 foodborne outbreaks reported during 1998 to 2007, 106 (1%) involved multiple states with a common source and were responsible for 8,786 (4%) illnesses, 1,310 (17%) hospitalizations, and 57 (29%) deaths. The most commonly reported etiologies were *Salmonella*, with 55 (52%) outbreaks and 4,735 (54%) illnesses, and Shiga toxin-producing *Escherichia coli*, with 30 (28%) outbreaks and 1,132 (13%) illnesses. Among 84 (79%) outbreaks (5,604 illnesses) that implicated a food that could be classified into a single commodity, the most commonly identified food commodities were beef, with 22 (26%) outbreaks and 770 (14%) illnesses; leafy vegetables, with 13 (16%) outbreaks and 1,216 (22%) illnesses, and fruits-nuts, with 12 (14%) outbreaks and 721 (13%) illnesses; pathogen-commodity pairs responsible for the most outbreak-related illnesses were *Salmonella* in vine-stalk vegetables, *Salmonella* in fruits-nuts, Shiga toxin-producing *Escherichia coli* in leafy vegetables, *Shigella* in leafy vegetables, and Shiga toxin-producing *Escherichia coli* in beef.

Significance: Large scale, multistate outbreaks provide data on sources of contamination because food items are usually contaminated before the point of preparation. Our data suggest that prevention strategies should place emphasis on *Salmonella* contamination of vine-stalk vegetables and fruits-nuts, and on Shiga toxin-producing *Escherichia coli* contamination of leafy vegetables and beef.

P3-46 Epidemiological Approaches for Food Safety

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Introduction: Incidence of foodborne disease (FBD) is difficult to estimate; however, foodborne pathogens cause approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths annually in the US. Treatment of FBD creates a substantial burden on the health care system, loss of productivity and economic burden is estimated at $20 to $40 billion annually. In 1996, active surveillance began for laboratory confirmed cases of nine selected foodborne pathogens in ten states. Several multi-state population-based surveillance studies have indicated an increase in prevalence of these foodborne pathogens, particularly *Campylobacter*, *Salmonella*, STEC O157, *Shigella, and Vibrio.* It is common knowledge that there is underreporting; although FBD can be severe or fatal, milder cases are often not detected through routine surveillance. *Staphylococcus aureus* infections, though not under active surveillance, are also on the rise, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA).

Purpose: The objectives of this study were to examine and evaluate risk factors for FBD, and to quantify the effect of FBD from outbreaks statistics.

Methods: Data sources for analysis include but are not limited to the Foodborne Diseases Active Surveillance Network (FoodNet), the National Notifiable Disease Surveillance System, the National Hospital Ambulatory Medical Care Survey, National Hospital Discharge Survey and Active Bacterial Core Surveillance (ABCs) Report.

Results: Results indicated that during 1996 to 2006, the leading cause of FDB outbreaks were *Salmonella* spp. (1,183), *Escherichia coli* spp. (259), *Campylobacter* spp. (136), *Shigella* spp. (122) and *Staphylococcus* spp. (167). The pathogen resulting in the highest number of death was *Listeria monocytogenes* (125/100,000 individuals). Children < 1 to 10 years were more susceptible to *Salmonella*, *Shigella* and *Campylobacter*, 30-<40 had a higher prevalence of *Campylobacter* spp. infection while those > 60 have higher incidence of listeriosis.

Significance: The paper concludes that public knowledge/perception of food safety, handling, and risks as related to food consumption is crucial in reducing the trend of food related diseases.

P3-47 Ethnic Food Safety Trends in the United States: CDC Foodborne Illness Data from 1990 to 2006

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Introduction: Ethnic foods are popular in the United States. The Agricultural Marketing Resource Center estimated that annual sales of ethnic foods will reach 75 billion dollars over the next decade. However, limited food safety knowledge is available for some of those ethnic foods.

Purpose: This study examined foodborne illness data relating to ethnic foods reported to the Centers for Disease Control and Prevention (CDC) from 1990 through 2006.

Methods: Foodborne illness data obtained from the CDC were examined and categorized in order to determine food safety trends. Ethnic foods were classified into three major groups: Mexican, Italian, and Asian foods. Total numbers of outbreaks and cases for all ethnic foods were compared to the total numbers of foodborne illness outbreaks and cases.

Results: Foodborne illness outbreaks associated with the three classes of ethnic foods were 6.4% of total foodborne diseases in 1990 and 8.1% in 2006. These foodborne disease cases showed no trend over time. The largest numbers of outbreaks were reported in Florida (n = 301), California (n = 218), Washington (n = 84), New York (n = 72), and Maryland (n = 70). The majority of incidents were associated with restaurants (51%), followed by private homes (20%), other (15%), workplaces (6%), schools (5%), and churches/temples (3%). The majority of the outbreaks in ethnic foods were caused by unknown pathogens, followed by Norovirus (10%), *Salmonella* spp. (5%), *Clostridium perfringens* (5%), and others (10%). Further, different ethnic foods have different microbial profiles. The major organisms specifically implicated Italian, Mexican, and Asian foods, respectively, were Norovirus (38%), *Salmonella* spp. (26%), and Norovirus (50%).

Significance: These findings will increase awareness among food safety professionals so that risks due to these foods can be determined and minimized.

P3-48 Restaurant Outbreaks 1990–2006

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Introduction: The Center for Science in the Public Interest (CSPI) maintains a database of foodborne illness outbreaks that have been linked to specific foods. CSPI identified a total of 5,778 outbreaks of illness linked to specific foods, involving 168,898 individual illnesses that occurred between 1990 and 2006.

Purpose: The purpose of this study was to analyze the illnesses linked to restaurant outbreaks between 1990 and 2006.

Methods: As outbreaks can range in size from 2 to over a thousand persons, the best tool for comparison is illnesses linked to outbreaks, with each illness equal to one person. Using this technique, CSPI researchers compared foodborne illness outbreaks linked to restaurants to those linked to private households and found that restaurant outbreaks were nearly twice as common as private household outbreaks, and restaurant outbreaks affected nearly four times as many people as those in private households.

Results: CSPI found that forty-one percent (2,388) of outbreaks identified in CSPI's database were attributed to restaurants, including 32% (53,399) of the total illnesses. Twenty-two percent (1,250) of outbreaks were attributed to private household, including 8.6% (14,551) of the total illnesses. The specific food categories with the greatest number of illnesses were produce (29%), multi-ingredient foods without meats such as salads, pizza, and sandwiches (17%), multi-ingredient foods with meats (11%), eggs (9%), seafood (8%), poultry (8%), and beef (7%). The most common pathogens implicated in restaurant outbreaks were *Salmonella* (38%), Norovirus (27%), *Clostridum* (9%), *E. coli* (5%) and *Shigella* (5%).

Significance: This analysis showed that over two-thirds (69%) of illnesses linked to restaurant outbreaks were caused by non-meat foods such as seafood, multi-ingredient foods without meats, eggs, produce, dairy, breads, and beverages. Twenty percent were attributed to meats, such as beef, poultry, pork, and luncheon meats, and 11% were multi- ingredient foods or meals containing meat.

P3-49 Relative Rates of Illnesses by Food Category, Adjusted for Consumption, 1999 to 2006

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Introduction: The Center for Science in the Public Interest (CSPI) maintains a database of foodborne illness outbreaks that have been linked to specific foods. CSPI identified a total of 5,778 outbreaks of illness linked to specific foods, involving 168,898 individual illnesses that occurred between 1990 and 2006.

Purpose: The purpose of this study was to analyze the illnesses linked to outbreaks in CSPI's database for selected food categories, and compared this data to consumption data for the years 1999 to 2006, to reflect current trends.

Methods: As outbreaks can range in size from 2 to over a thousand persons, the best tool for comparison is illnesses linked to outbreak, with each illness equal to one person. CSPI researchers analyzed the illnesses linked to outbreaks for each food category, and compared it to consumption data for the years 1999 to 2006, to reflect current trends. To standardize its analysis, CSPI choose dairy products to reflect a nearly fully-pasteurized product. The data sources used were from USDA Economic Research Service for consumption data. The population estimates were from the US Census Bureau. CSPI provided the food attribution estimates, using data provided by the Centers for Disease Control and Prevention.

Results: CSPI found that compared to dairy, reported illnesses were four times more frequent from produce, eight times more frequent from pork, eleven times more frequent from beef, thirteen times more frequent from eggs, fifteen times more frequent from poultry, and twenty-nine times more frequent from fish and shellfish.

Significance: The study showed current trends of relative rates of illnesses linked to outbreaks among selected food categories when adjusted for consumption during the period of 1999 to 2006. Acccording to this analysis, fish and shellfish topped the list to cause more sicknesses per pound than any other food category. After dairy, produce is the second safest category of food, followed by pork. Instead of relying on recalls and warnings, the Food and Drug Administration should focus on preventing these problems from ever reaching consumers. Modernizing the food safety system should be an urgent priority to reduce outbreaks and illnesses from food and restore consumer confidence.

P3-50 Beef Grinding and Record Keeping Practices, a Survey of Retail Establishments in Three States, 2008

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Introduction: Ground beef has been implicated as a transmission vehicle in outbreaks of disease caused by *Escherichia coli*, *Salmonella*, and other foodborne pathogens. Traceback of contaminated beef to the slaughter facility is often unsuccessful because of inadequate recordkeeping at retail establishments that grind beef products.

Purpose: To describe beef grinding and record keeping practices at retail establishments.

Methods: Environmental Health Specialists Network (EHS-Net) personnel in three states (California, Minnesota, Tennessee) surveyed a convenience sample of retail establishments that grind beef. In each establishment that kept grinding logs, three randomly selected records were reviewed to determine whether data elements critical for traceback investigations were completed.

Results: We surveyed 124 stores, of which 60 (49%) kept grinding logs, including 54 (74%) of 73 chain and 6 (12%) of 51 independent stores. One hundred seventy-six grinding records were reviewed. These establishments ground beef a median of seven times per week (range 2 to 140), and ground a median of 40 pounds (range 2 to 800) per grind. Seventy-three percent of the records included the establishment code of the source beef, 72% included the grind date and time, and 59%

included the lot number of the source beef. Seventy-five percent of records noted whether trimmings were included in grinds, and 57% had documented clean-up. Only 39 (22%) records had all of these variables completed. In stores that did not keep grinding logs, 40% of were unaware of their purpose.

Significance: Half of retail establishments that grind beef products maintained logs of their grinding practices, and only 22% of records had complete information. During outbreaks associated with ground beef products, regulatory agencies need detailed and accurate information about the source and types of products included at the point of final grinding. For conducting effective and efficient traceback investigations, retail establishments should maintain better records of all grinding activities.

P3-51 Effect of Various Antimicrobials on the Growth Kinetics of Foodborne Pathogens in Ready-to-Eat Pyeonyuk (Boiled and Pressed Pork)

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Introduction: Vacuum packaged Pyeonyuk (boiled and pressed pork) was recently introduced as a convenience food in Korea and is often sold at room temperature. Pyeonyuk contaminated with *Salmonella* or *Staphylococcus* have been reported, and control measure of these pathogens in Pyeonyuk is needed.

Purpose: The objective of this study was to compare the antimicrobial effects of garlic, potassium lactate and sodium diacetate(PL/SDA), ε-polylysine, and potassium sorbate on the growth of *S.* Typhimurium and *S. aureus* in RTE Pyeonyuk at various temperatures.

Methods: Pyeonyuk was prepared with 4% garlic, 3% PL/SDA, 1.5% ε-polylysine or 0.2% potassium sorbate. *S.* Typhimurium and *S. aureus* were inoculated into thin slices of Pyeonyuk and growth kinetics of *S.* Typhimurium and *S. aureus* were evaluated at various temperatures. The concentration of each antimicrobial was obtained from the result of our previous broth study to control the growth of *S.* Typhimurium and *S. aureus* in Pyeonyuk.

Results: Generally, the lag time (LT) of *S.* Typhimurium was longer than that of *S. aureus* at 17, 24 and 30°C, regardless of the kinds of antimicrobial agents used. Growth of *S.* Typhimurium and *S. aureus* was not observed at 10°C in Pyeonyuk made with 3% PL/SDA or 1.5% ε-polylysine. LT of *S.* Typhimurium with 4% garlic, 3% PL/SDA, 1.5% ε-polylysine or 0.2% potassium sorbate, were 5.5, 6.3, 15.8, 11.6 and 6.7 hrs, respectively at 24°C. LT of the corresponding control was 5.5 hrs. These data suggest that the addition of 3% PL/SDA or 1.5% ε-polylysine in RTE pyeonyuk effectively controlled the growth of *S.* Typhimurium and *S. aureus* at room temperature. On the other hand, 0.2% of potassium sorbate, which was the permitted level for meat products, did not inhibit the growth of *S.* Typhimurium and *S. aureus* in the present study.

Significance: Addition of 3% PL/SDA and 1.5% ε-polylysine to Pyeonyuk can be recommended for the safety of RTE Pyeonyuk sold in convenience stores. The obtained data can be used to develop growth models to predict the growth of *S.* Typhimurium and *S. aureus* in Pyeonyuk as a function of temperature and antimicrobial agents.

P3-52 Effectiveness of Different Antimicrobial Treatments on Microbial Populations on Alligator Carcasses

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Introduction: Alligator meat is mainly consumed in the southern United States; however, the industry wants to expand its market. To take advantage of the potential for increased market penetration and industry viability, the industry is also aware that the final product quality of alligator meat needs improvement.

Purpose: The purpose of this study was to evaluate the effects of different antimicrobial agents on alligator meat and to identify effective treatments.

Methods: Four-month-old alligators were skinned and gutted, and the carcasses were treated individually with different antimicrobial agents dissolved in an ice water bath for 5 min in order to find the most effective treatment. The antimicrobial agents were lactic acid (200 ppm), sodium benzoate (200 ppm), calcium lactate (200 ppm), chlorinated water (150 ppm of sodium hypochlorite), and acidified sodium chlorite (ASC) (50 ppm). The two most efficient antimicrobial agents were lactic acid and ASC combined with steam (60 s at 2 to 3 in. from surface). For the combined treatments, the samples were steamed before soaking in the antimicrobial solutions. The back, tail and ribs of alligator carcasses were swabbed (2 in^2) and then analyzed for total coliforms, total Enterobacteriaceae, *Escherichia coli* and *Salmonella* spp.

Results: Treatments with lactic acid, calcium lactate, chlorinated water and the combinations of steam and Lactic Acid or ASC, significantly reduced total coliforms, total Enterobacteriaceae and *Salmonella* spp. counts by 1 log from control levels. Sodium benzoate did not show significant reduction of any of the bacteria analyzed. The combination of steam and ASC was the treatment that proved to be the most effective in reducing coliforms, Enterobacteriaceae and *Salmonella* spp. on alligator carcasses.

Significance: The results of this study can help the alligator industry to increase their yield and extend the shelf life of their products.

P3-53 Growth of *Listeria monocytogenes* on Three Ham Products Formulated with and without Potassium/Sodium Lactate and Sodium Diacetate

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Introduction: Data on the growth potential of *Listeria monocytogenes* on commercially available Ready-to-Eat meat products are needed for improvement or re-evaluation of current quantitative risk assessments of the pathogen in these products, or for development of new risk assessments.

Purpose: This study evaluated the growth of inoculated *L. monocytogenes* on slices of three ham products from different manufacturers, each formulated with and without antimicrobials, during vacuum-packaged storage.

Methods: Commercially manufactured ham products formulated with and without antimicrobials were obtained from company A (product X) and B (products Y and Z). Ham formulated with antimicrobials contained 1.5% potassium lactate plus 0.05% sodium diacetate (product X) or 1.44% sodium lactate plus 0.1% sodium diacetate (products Y and Z). Products were sliced (0.1 to 0.2 cm thick) and cut (5 × 5 cm), inoculated (1-2 log CFU/cm^2) with a 10-strain composite of *L. monocytogenes*, vacuum-packaged (two slices/sample) and stored at 4, 7 or 12°C. Samples were periodically analyzed (two replications/three samples per treatment) for *L. monocytogenes* (PALCAM agar) and total microbial (Tryptic Soy Agar with 0.6% yeast extract) populations for up to 90 days, depending on the product type and temperature. Lag phase durations, growth rates and maximum growth levels of *L. monocytogenes* on each of the products were calculated by fitting the growth curves with the Baranyi model.

Results: Lag phase durations of *L. monocytogenes* on all ham products formulated without antimicrobials and stored at all temperatures were 0 to 2.9 days. As expected, growth rates of the pathogen on these products were lower when samples were stored at 4°C (0.19-0.26 log CFU/cm^2/day) than at 12°C (0.67 to 0.95 log CFU/cm^2/day); however, in all cases, growth to maximum levels of 6.5 to 7.9 log CFU/cm^2 were obtained on all products without antimicrobials. Inclusion of antimicrobials in ham formulations extended the lag phase of *L. monocytogenes*; lag times at 4°C were up to 29.2, 5.2 and 22.2 days on products X, Y and Z, respectively. Growth rates of the pathogen on all products with antimicrobials were 0.06-0.14 (4°C), 0.06 to 0.17 (7°C) and 0.17 to 0.38 (12°C) log CFU/cm^2/day.

Significance: These data may be valuable to risk assessors when developing or updating risk assessments of *L. monocytogenes* on Ready-to-Eat meat products.

P3-54 DSC Weibull Type Distribution of Resistances of *Escherichia coli* to Different Concentrations of Potassium Sorbate and Sodium Benzoate

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Introduction: Weibull distribution applied to survival data is an interesting and useful model to explain microbial behavior under inhibition or inactivation conditions.

Purpose: The main objective of our research was to evaluate and model the survival of *Escherichia coli* ATCC 35218 in tripticase soy broth (TSB) formulated with several concentrations (1000, 2000, up to 7000 ppm) of potassium sorbate or sodium benzoate.

Methods: TSB formulated with each antimicrobial type and concentration was inoculated with 10^8 UFC/ml *E. coli* from stationary phase. *E. coli* survivors were monitored by plating 10-fold dilutions in tripticase soy agar (TSA) each hour during the first 10 h and then every 4, 6 or 8 hours (depending on the antimicrobial concentration) up to 48 h. Inoculated plates were incubated at 35°C for 24 h and the survivors were counted. In order to quantify the microbial response in every studied condition, the Weibull type distribution of resistances model was applied. Model parameters were obtained using nonlinear regression.

Results: In general, both antimicrobials were effective suppressing *E. coli* growth; increasing antimicrobial concentration reduced the time to reach an 8 log cycle reduction. Experimental curves were highly correlated to predicted data, obtaining significant determination coefficients. *E. coli* survival curves in media with lower antimicrobial agent concentrations showed downward concavity ($n > 1$), and with higher concentrations exhibited upward concavity ($n < 1$). The increase in antimicrobial concentration reveals differences in the distribution of resistances. In general, when potassium sorbate was added the distribution of resistances showed more marked right-skewed curves than the observed curves for sodium benzoate, indicating that the majority of the organisms were destroyed in a short time after their exposure to potassium sorbate.

Significance: The Weibull type distribution model was useful to explain observed differences between antimicrobial agents. The use of potassium sorbate was in general more effective than sodium benzoate in activating *E. coli*.

P3-55 Bactericidal Efficacy of Salicid (Low Concentration of Electrolyzed Water) on Different Foodborne Pathogens

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Introduction: Numerous sanitizers have been examined for their effectiveness in killing or removing pathogenic bacteria. Recently, electrolyzed water has become a notable antimicrobial agent for its effective bactericidal activity and lack of residual effect on food. This is the first study that has been done on Salicid as a new device, producing sanitizer solution containing a low concentration of hypochlorous acid (2~5 ppm) that can be used in the food industry.

Purpose: This study was designed to evaluate the efficacy of physiological saline solution produced from salicid device for inactivation of different types of foodborne pathogens.

Methods: The efficiency of salicid (pH 6.7, ORP 760 mv, residual chlorine 2 and 5ppm) for inactivation of pure cultures of different foodborne pathogens (*Escherichia coli* O157:H7 ATCC 43894, *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 12488 and *Salmonella* Typhimurium ATCC 14028) with six exposure times (30 s, 1 min, 3 min, 5 min, 7 min, 10 min) was determined. A dipping method (dipping temp. 22 ± 20°C) was followed for this study. Log reduction per test material, contact time and organism were recorded.

Results: Compared to the untreated control a reduction of 2.30 to 6.08 log CFU/ml for different foodborne pathogens was seen as the result of treatment with salicid. The highest bactericidal effect was found with *Staphylococcus aureus*

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for 5 ppm salicid with 30 s immerse time. Salicid treatment (dipping time 30 s and 5 ppm residual chlorine) also reduced *E. coli* O157:H7, *L. monocytogenes* and *S*. Typhimurium by 4.46, 4.85 and 4.71 log CFC/ml respectively. Our findings showed that in each pathogen, efficacy of salicid decreased slightly with increased dipping time, from 30 s to 10 min. There is no significant difference ($P > 0.05$) found between 2 and 5 ppm salicid treatment although sanitizing effect was always higher with 5 ppm salicid.

Significance: These results indicated that salicid is a most cost effective anti-microbial solution that does not produce the secondary pollutants and leaves no residues, because of low concentration of residual chlorine. Thus, salicid could be used in the food sanitation effectively against different foodborne pathogens.

P3-56 Acidic Calcium Sulfate as a Secondary Barrier to Control Post-extrusion *Salmonella* Contamination in Dry Pet Foods

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Introduction: Extruded pet foods are Ready-to-Eat products and are adulterated if contaminated with pathogens. National recalls due to *Salmonella* contamination, with associated human illnesses from handling, have prompted manufacturers to evaluate current processing methods and incorporate control measures. Extrusion processing renders pre-dried kibbles free of vegetative pathogens; however, downstream processing steps provide for potential surface recontamination from environmental sources.

Purpose: This study evaluated the effectiveness of acidic calcium sulfate (ACS; pHresh 10™, 10 N) in pre-extrusion kibble formulations and subsequently applied during coating as a secondary *Salmonella* barrier.

Methods: Three basic kibble formulae containing 0 (pH 5.81), 0.8 (pH 5.26), and 1.3 percent ACS (pH 4.99) were extruded and dried according to standard commercial protocols. Kibbles were misted with a 5-serovar mixture of *Salmonella* (2 log CFU/g) prior to coating to simulate post-extrusion contamination during drying. Each inoculated kibble formula was coated with choice white grease (6 ± 1percent final kibble moisture) containing 0, 0.5 and 1.5 percent ACS, resulting in final pH values of 5.7 to 5.9, 5.2 to 5.3 and 5.0-5.1, respectively. Coated kibbles were mist inoculated again to a final *Salmonella* level of 1.9 log CFU/g (simulating post-coating contamination), stored ambiently in bags, and analyzed after 0, 8, 24 and 48 h.

Results: Results demonstrated that kibbles containing 1.3 percent ACS immediately reduced *Salmonella* counts by 0.4 to 1.3 logs; however, a large amount of injury as opposed to lethality was observed. After 8 h storage, the *Salmonella* population in these kibbles was further reduced to virtually non-detectable levels with little injury detected when coatings contained 1.5 percent ACS. Comparable kibbles containing only 0.8 percent ACS also resulted in up to 1.6 log reductions regardless of coating formula; however, more injury was observed.

Significance: ACS eliminated moderate levels of post-extrusion *Salmonella* contamination when used in combination as a kibble ingredient and in coating solutions. ACS would provide dry pet food manufacturers a reasonable margin of protection against low-level post-extrusion contamination. Product palatability and nutritional/health impacts on pets were not evaluated in this study.

P3-57 *In vitro* Inhibition of *Listeria monocytogenes* with Acidic Calcium Sulfate Combined with Nisin or ε-Polylysine DSC

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Introduction: Listeria monocytogenes persists as a challenge to the safety of Ready-to-Eat (RTE) meat products. The pairing and application of food antimicrobials with differing mechanisms of inhibition but with compatible solubility and activity profiles may result in enhanced inhibition of *L. monocytogenes* on the surfaces of RTE meats.

Purpose: The purpose of the study was to evaluate the *in vitro* inhibition of *L. monocytogenes* exposed to the anti-microbial acidic calcium sulfate (ACS) alone or in combination with nisin (NIS) or ε-polylysine (EPL).

Methods: A checkerboard assay was utilized to determine the minimum inhibitory concentration (MIC) of *L. mono-cytogenes* strains (Scott A, 310, National Animal Disease Center (NADC) 2783 and 2045) exposed to NIS, EPL, and ACS singly or in combination. Strains were diluted to deliver 5.0 log CFU/ml at the outset of the experiment. The MIC was defined as the lowest concentration of the antimicrobial that resulted in a < 0.05 change in optical density at 630 nm (OD630) following 24 h of aerobic incubation at 35°C in Tryptose Phosphate Broth (pH 7.3). Antimicrobials were then tested in combination (ACS+NIS, ACS+EPL) and fractional inhibitory concentration (FIC) index values determined. The FIC index was defined as the sum of the FIC of antimicrobial A and antimicrobial B. Antimicrobial FIC values were calculated as the MIC of antimicrobial A in combination (concentration of A that produced inhibition across all concentrations of antimicrobial B) divided by the MIC of antimicrobial A applied singly. Antimicrobial interactions were defined as synergistic, additive, or antagonistic when the FIC index was < 0.5, 0.5 to 2.0, or > 2.0, respectively.

Results: The MIC for NIS was 3.13 ppm for *L. monocytogenes* Scott A and 6.25 ppm for *L. monocytogenes* NADC 2783 and 2045. The MICs for EPL were 6.25 ppm for *L. monocytogenes* Scott A and 12.50 ppm for *L. monocytogenes* NADC 2783 and 2045. ACS MIC for all strains was 1.25% (v/v). Combination FIC index values for NIS + ACS were 1.5, 1.0, and 1.0 for *L. monocytogenes* Scott A, NADC 2783, and 2045, respectively. Combination FIC index values for EPL + ACS were 1.5, 1.5, and 2.0 for *L. monocytogenes* Scott A, NADC 2783, and 2045, respectively.

Significance: Results indicate that the paired application of ACS with the antimicrobials NIS and EPL results in additive-type inhibition of *L. monocytogenes in vitro*. Combined food antimicrobials may provide enhanced pathogen inhibition as compared to application of a single antimicrobial. Further studies are needed to characterize the efficacy and interactions of antimicrobials on surfaces of RTE meat products.

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P3-58 Inappropriate Use of D-values for Determining Biocidal Activity of Various Antimicrobials

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Introduction: Decimal reduction values (D-values) are commonly used to illustrate microbial survival of various treatments and are defined as the time required to reduce the microbial population one logarithmic unit. The D-value can be calculated in three ways:, a linear regression, an end point calculation or an average of multiple endpoint calculations. The assumption made in calculating a D-value is that the rate of kill follows first-order kinetics under specified treatment conditions.

Purpose: The objective of this study was to investigate the application of established D-value calculations to survival curves for various bacteria, using the following antimicrobials: acidified sodium chloride, triclosan, octanoic acid and sodium hypochlorite.

Methods: Each antimicrobial solution was challenged with approximately 10^6 to 10^8 CFU/ml of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhi, and *Escherichia coli* independently and in triplicate. Test systems were sampled at each of the ten time points over a period of seven minutes, neutralized, pour plated then incubated at 35°C for 48 h (AOAC method 960.09). Survival curves were calculated using the log-transformed data with the Regression Analysis procedure, using SPSS.

Results: Correlation coefficients for all linear regression analyses ranged between 0.219 and 0.982, with only 3 of the 16 different treatment systems having an R^2 value above 0.9. Methods used for calculating D-values should lead to the same result if the survival curve in a given condition is linear. The calculated D-values were different using end point analysis (Stumbo method), linear regression and inactivation curve average.

Significance: This study demonstrates the non-linearity of inactivation curves of antimicrobials. D-value estimations cannot be reliably used to illustrate biocidal activity in antimicrobial test systems.

P3-59 Antimicrobial Activity of Various Natural Compounds against *Escherichia coli* O157:H7 Cultured in Ground Beef Extract

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Introduction: Moisture-enhanced beef products contaminated with *Escherichia coli* O157:H7 have been involved in human illness. Considering consumer preference for natural antimicrobials, their potential for use in meat enhancement solutions should be evaluated.

Purpose: This study screened various natural ingredients, alone or in combinations, for their potential to control *E. coli* O157:H7 in meat extract, in combination with common salt and phosphate.

Methods: Beef knuckles (100 g) were blended with sterile distilled water (200 ml), and the mixture was filtered through cheese cloth, the extract was dispensed into test tubes (10 ml). Sodium chloride (NaCl; 0.5%) and sodium tripolyphosphate (STP; 0.25%), commonly present in meat enhancement solutions, were added along with each of the following, alone or in combination: cetylpyridinium chloride (CPC; 0.02% and 0.04%), sodium diacetate (SD; 0.25%), lactic acid (LA; 0.3%), thyme oil (TO; 0.01%, 0.05%, 0.1%, 0.25%, and 0.5%), grapefruit seed extract (GSE; 0.01%, 0.1%, 0.25%, 0.5%, and 1.0%), and basil essential oil (BO; 0.01%, 0.25%, and 0.5%). The samples were inoculated (3 log CFU/ml) with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7 and incubated at 15°C for 48 h (2 replicates, 3 samples/treatment/replication). Counts were determined with Tryptic Soy Agar (TSA) and TSA with rifampicin (100 µg/ml).

Results: At day-0, CPC (0.04%), TO (0.25% and 0.5%) and GSE (0.5% and 1.0%), alone or in combination with other ingredients, reduced *E. coli* O157:H7 by approximately 3 log CFU/ml, whereas BO (0.5%) singly or BO (0.5%) with SA reduced pathogen counts by 1 log-cycle after 48 h incubation. Additionally, SD, LA, GSE (0.25%), BO (0.1 and 0.25%), and TO (0.1%), alone or in combination with other ingredients, showed bacteriostatic effects against *E. coli* O157:H7 after the 48 h incubation period. The background flora was more resistant to the antimicrobials and/or grew faster than *E. coli* O157:H7; however, it was found to be susceptible to TO (0.25% and 0.5%), GSE (0.5% and 1.0%), and BO (0.5%) alone or in combination with other ingredients, and to CPC (0.04%) with BO (0.25%).

Significance: Ingredients such as grapefruit seed extract, basil essential oil, thyme oil, and cetylpyridinium chloride were effective against *E. coli* O157:H7 in meat brine solutions, and may be considered for potential use in non-intact meat products.

P3-60 Potential Food Application of Plant Derived Peptides That Inhibit the Growth of Spoilage and Foodborne Bacteria

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Introduction: The microbial safety of fresh and fresh-cut produce requires development of novel strategies to ensure the safety of products and health of consumers. Chemical sanitizers presently used offer limited reduction in microbial populations associated with fresh and fresh-cut produce and are restricted to use during certain steps in the production process. The use of natural antimicrobial compounds may provide an effective alternative and have greater consumer acceptance.

Purpose: This study determined the antimicrobial activity of three plant derived peptides (RD1, RD2, and RD3) against *Escherichia coli* O157:H7, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria monocytogenes*.

Methods: Inhibitory peptides ranging in size from 63 to 20 amino acids were synthesized based on previously available sequence information. Bacteria were culture in LB broth to achieve a concentration of 10^5 CFU/ml. The minimum inhibitory concentration of each compound against each organism was determined using the microbroth dilution

assay. Each concentration was tested in triplicate (three wells/concentration) and the assay was conducted twice. The concentration of each peptide tested ranged from 2,000 to 1.95 µg/ml. On-going study: Lettuce pieces (2 × 2 cm) were submerged in a suspension containing 10^6 CFU/ml of a target organism. The lettuce pieces were allowed to dry and then immersed in suspensions of RD1 or RD3. The lettuce pieces were processed to determine change in population of the target organism.

Results: At 250 µg/ml RD1, *Salmonella* was effectively inhibited. However, 1,000 µg/ml of RD1 was required to inhibit *E. coli* O157:H7 and *P. aeruginosa*. The Gram-positive pathogens were not effectively inhibited by RD1. *L. monocytogenes* and *S. aureus* were effectively inhibited by 1,000 µg/ml RD3. Definitive inhibition of pathogen growth was not demonstrated for RD2. Experiments demonstrated that 1,000 µg/ml RD1 and RD3 are most effective in inhibiting growth of bacteria used in the present study. Experiments designed to determine effectiveness of killing target bacteria associated with lettuce have not been completed for all bacteria.

Significance: The research provides a starting point for the utilization of plant derived antimicrobials to control foodborne pathogens associated with fresh and fresh-cut produce. The antimicrobial compounds could potentially be over-expressed in food crops (e.g., lettuce), thereby protecting the crop, or in plants providing a cost effective approach for production of the natural antimicrobial agents.

P3-61 Antimicrobial Activity of Recombinant Tobacco Osmotin

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Introduction: Pathogenesis-related proteins (PR) are produced by plants in response to environmental stress, such as pathogen infections and extremes of salinity. Due to their nature and function in host plants, there is a great potential for some PR proteins to be used in controlling foodborne pathogens. Among PR proteins, osmotin from tobacco (*Nicotiana tobacum*) is one of the most studied for antimicrobial activity.

Purpose: The purposes of this study were to 1) develop an appropriate system for expressing the tobacco osmotin in *E. coli*, 2) establish an effective protocoal for expressed osmotin purifucation, and 3) test its antimicrobial activity against bacteria and fungi.

Methods: The PCR product of mature tobacco osmotin gene coding sequence was inserted in pET20b, and the ligation mixture was transformed into *E. coli*. Sequence-confirmed plasmids were transformed into DE3 strain of *E. coli*, Rosetta2 pLYS, for the production of recombinant tobacco osmotin. The expressed tobacco osmotin in inclusion bodies was dissolved in 8 M urea and purified through CM cellulose and nickel-aggarose columns. Antimicrobial activity of recombinant tobacco osmotin was tested against 7 yeasts and 5 bacteria.

Results: The recombinant tobacco osmotin was induced with lactose or IPTG in ZYM 505 medium at late log phase of the culture (O.D.600 nm 2 to 4) at pH 6.8 to 7.0 for large scale production and remained stable in the inclusion body. This protein was denatured in 8 M urea and highly purified proteins were obtained by using CM-cellulose and cobalt-agarose columns. Recombinant tobaco osmotin can inhibit the growth of *Cryptococcus neoformans*, *Candida albicans*, *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*, *Pichia methanolica*, *Staphylococcus aureus* and *Vibrio cholerae*.

Significance: Osmotin eixsts naturally in many crops and theoretically is safe for human consumption. The recombinant tobacco osmotin has great potential for use in controlling foodborne pathogens.

P3-62 The Effect of Chitosan on the Infectivity of Murine Norovirus, Feline Calicivirus and MS2 Bacteriophage

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Introduction: Chitosan is known to have inhibitory effects on microorganisms of concern to plants, animals and humans. However, the effect of chitosan on human enteric viruses of public health concern has not been extensively investigated. Human noroviruses are the leading causes of nonbacterial gastroenteritis worldwide and remain persistent in the environment. Alternative technologies and novel approaches to control their spread are much needed. In the absence of standard cell-culture based infectivity assays for human noroviruses, surrogates are used to determine their infectivity.

Purpose: The purpose of this study was to determine the effect of two types of chitosan on the infectivity of three surrogates of human enteric viruses, murine norovirus (MNV-1), feline calicivirus (FCV-F9), and MS2 (ssRNA) bacteriophage.

Methods: Chitosan oligosaccharide lactate (M.Wt.<5kDa) and water-soluble chitosan (M.Wt.50 kDa) were each dissolved in water at concentrations of 1.4%, 0.7%, and 0.35%, mixed with equal volume of virus (MNV at ~7 log PFU/ml, FCV at ~8 log PFU/ml, or MS2 at ~8 log PFU/ml) and incubated at 37°C for 2 h. Infectivity of each treated virus was evaluated twice, using standardized plaque assays, in comparison to untreated virus controls. MNV and FCV were assayed in 6-well plates using RAW 264.7 and Crandell Reese Feline Kidney (CRFK) cells, respectively, and MS2, using its *E. coli* host by the double overlay method.

Results: The two chitosans showed varied results for viral inactivation. The chitosan oligosaccharide showed no effect on the infectivity of all three surrogates. Interestingly, the water-soluble chitosan at 0.7% decreased FCV titers by ~2.7 log PFU. Its antiviral effect on FCV decreased with decreasing concentration. The water-soluble chitosan also decreased MS2 infectivity by ~1.3 log PFU regardless of the concentration used. MNV was found to be resistant to both chitosan treatments at the concentrations studied. The use of higher molecular weight chitosan, and chitosan at higher concentrations with longer incubation times, may be necessary to inactivate MNV. For FCV inactivation, shorter incubation times with chitosan will need to be investigated.

Significance: These results show promise for the inactivation of human enteric virus surrogates by chitosan for its potential application in the food environment.

P3-63 Extracts of *Agave americana* Demonstrate Activities against Conidiogenesis and Aflatoxin Production by *Aspergillus parasiticus*

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Introduction: Since consumers demand safe commodities, and because of enhanced public awareness of the dangers of many synthetic fungicides, the importance of investigating alternative, natural products to control mycotoxigenic fungi is clear.

Purpose: This study investigated the effect of aqueous extracts of *Agave americana* on growth, aflatoxin production, and the expression of the aflatoxin pathway regulatory gene aflR of *A. parasiticus*.

Methods: A. parasiticus strains SRRC 148, SRRC 143 (Su-1), and *A. parasiticus* SRRC 162, a mutant (nor-) that accumulates norsolorinic acid (NOR), were inoculated into A&M liquid medium; then plant extracts were added, and the mixture was incubated at 28°C for 7 days. Aflatoxin and norsolorinic acid were assayed by HPLC and spectrophotometry, respectively.

Results: While the extract of *A. americana* did not exhibit an inhibitory effect on growth of the studied fungi, conidiogenesis, norsolorinic acid accumulation (in the nor- mutant), and aflatoxin production were significantly affected. Further, Northern blot analysis indicated that the aqueous *Agave* extract did not affect transcription of aflR after 48, 72, or 96 h of incubation.

Significance: The results of our work indicate that extracts of *A. americana* could be used effectively to control aflatoxin contamination.

P3-64 Antimicrobial Activity of Greater Galangal (*Alpinia Galanga* (Linn.)) Flowers

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Introduction: Emerging multidrug-resistant microorganisms have raised global concern, and alternatives to conventional antibiotic treatments are greatly needed. Parts of the greater galangal plant have been used in traditional herbal medicine in Asia for centuries, and the antimicrobial activity of essential oils extracted from its rhizome is well established.

Purpose: This study investigated the potential antimicrobial activity of greater galangal's edible flowers (used in Thai cuisine) against common foodborne pathogenic bacteria, as well as the effects of drying methods and solvent types on the antimicrobial activity of galangal flowers.

Methods: Fresh galangal flower buds were oven- or freeze-dried and extracted with ethanol (190 proof) and hexane. The filtered extracts were concentrated to complete dryness under reduced pressure at 40°C using a rotary evaporator. The antimicrobial activity of the crude extracts against 5 pathogenic bacteria (*Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *Staphylococcus aureus*, and *Shigella*) was determined using an agar disc diffusion method. The size of the inhibition zone was measured in millimeters.

Results: Greater galangal flower extracts were effective against Gram-positive *Staphylococcus aureus* and *L. monocytogenes*. Oven-dried ethanolic extracts had the greatest antimicrobial efficacy against *Staphylococcus aureus* ATCC 35548 of all test extracts, with an inhibition zone of about 31 mm. In contrast, no antimicrobial activity was observed with *E. coli* O157:H7 and *Salmonella*. The type of solvent and the type of drying method also affected the antimicrobial efficacy of galangal flowers. Overall, oven-dried samples extracted with ethanol exhibited the most antimicrobial activity, and freeze-dried samples extracted with ethanol yielded the least antimicrobial activity.

Significance: Results confirmed our hypothesis about the antimicrobial activity of greater galangal flowers. This extract could be used as a food additive to inhibit the growth of common foodborne pathogens.

P3-65 Bactericidal Effects of Titanium Dioxide/UV Reaction on Foodborne Pathogenic Bacteria and Thermoduric Spores

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Introduction: A titanium dioxide (TiO_2) photocatalytic reaction under UV irradiation provides a high rate of disinfection. The TiO_2/UV photocatalytic reactions involve oxidations as UV-illuminated TiO_2 generates reactive oxygen species (ROS). They also show effective disinfection activities as the ROS generation causes structural disruption of bacteria and spores in a relatively short period.

Purpose: Bactericidal effects of TiO_2/UV photocatalytic reaction on foodborne pathogenic bacteria and thermoduric spores were investigated.

Methods: Escherichia coli O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium were treated with TiO_2/UV reaction and sampled at 10 s intervals for 90 s. *Bacillus cereus* spores and *Bacillus subtilis* spore were treated with TiO_2/UV reaction and sampled at 30 s intervals for 5 min. The structural and morphological changes in the bacteria and spores during the treatments were evaluated under a scanning electron microscope (SEM) and a transmission electron microscope (TEM). The production of ROS in spores was detected using the ROS-sensitive probe 2',7'-dichloro dihydro fluorescein diacetate (DCF-DA).

Results: TiO_2/UV photocatalytic treatments completely eliminated foodborne pathogenic bacteria within 80 s and spores within 300 s (initial counts of the bacteria and spores were in the range of 5 to 6 log CFU/ml). SEM and TEM images showed significant structural disruptions in the bacteria and spores within 3 min and 15 min, respectively. TiO2/UV photocatalytic reaction induced ROS generation in thermoduric bacterial spores, and DCF fluorescence of *B. cereus* and *B. subtilis* spores exhibited 54% and 213% increases in 15 min.

Significance: TiO_2/UV photocatalytic treatment can be used as an effective method to eliminate thermoduric spores as well as foodborne pathogenic bacteria. This technology can be usefully applied to the fresh produce industry.

P3-66 *Staphylococcus aureus* Inactivation Kinetics during Thermo-ultrasonication Treatments at Selected Amplitudes and with Different Vanillin Concentrations

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Introduction: Low frequency ultrasound can be included in the formulation of combined methods of food preservation to diminish the intensity of traditional factors. An efficient selection of factors depends on microbial inactivation kinetics.

Purpose: The combined effect of simultaneous application of heat (40, 50 or 60°C), low frequency ultrasound (20 kHz; at 60, 75 or 90 microns amplitude), and vanillin concentration (200, 350 or 500 ppm) on *Staphylococcus aureus* viability suspended in laboratory broth formulated at a_w 0.96 and pH 3.5 were evaluated following a Box Behnken experimental design.

Methods: Media adjusted to a_w and pH were inoculated with 10^6 CFU/mL. An ultrasonic horn (13 mm) was submerged into the broth, and then thermo-ultrasound was applied. Samples were taken periodically and survivors were determined by surface platting. Survival curves were adjusted with Fermi equation as a dose-response model.

Results: Surface response analysis demonstrated that individual and well as double interactions of the evaluated variables significantly ($P < 0.05$) affect *S. aureus* survival response. At higher temperatures, the predominant effect on *S. aureus* inactivation was mainly due to heat treatment, but as temperature was reduced the effects of vanillin concentration and wave amplitude were more important, obtaining comparable inactivation patterns for 60°C and for combining 40°C with higher amplitudes (90 µm) and 500 ppm vanillin concentration.

Significance: The simultaneous application of ultrasound, heat and vanillin concentration had a synergistic inhibitory effect on *S. aureus*. The combined processes must be further studied with other microorganisms prior to its possible industrial application.

P3-67 DSC Efficacy of BioSealed for Concrete™ against Multiple Strains of *Listeria* spp. and Their Biofilms on Concrete Surfaces

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Introduction: Listeria monocytogenes is an important foodborne pathogen that has been widely isolated from food processing facilities and has been implicated in numerous foodborne outbreaks, resulting in tremendous economic losses and human illnesses.

Purpose: In this study, antimicrobial efficiency of BioSealed for Concrete™ to prevent bacterial attachment and colonization of multiple strains of *Listeria* (*L. monocytogenes*, *L.* Scott A and *L. innocua*) on concrete cement blocks was evaluated.

Methods: Cement blocks were divided into four different treatment groups: A) No Biosealed application, B) Biosealed applied before inoculation, C) Biosealed applied after inoculation, or D) Biosealed applied before and after inoculation. The cultures were prepared by inoculating *Listeria* strains into brain and heart infusion broth (BHI) and incubating at 35°C for 24 h. Cement blocks were inoculated by submerging in BHI broths containing one of the three *Listeria* strains and incubated at 35°C for 24 h (ca. 10^9 CFU/ ml). External surfaces of the inoculated blocks were swabbed using sterile swabs and placed in 10 mL peptone water (PW). The cement blocks were broken in half and interior surfaces were swabbed to determine *Listeria* spp. levels. Following 24 h of incubation of Modified Oxford agar plates (MOX) at 35°C, survival of *Listeria* populations on internal surfaces varied among strains. Experiments were performed in triplicate and results were analyzed using SAS.

Results: On the external surface of cement blocks, significantly lower populations of all the three strains of *Listeria* ($P < 0.05$) were observed for treatments C and D when compared with treatments A and B, except for *L. monocytogenes*. When comparing treatments A and C, as well as groups B and D, were compared Biosealed was shown to be efficient for biofilm removal on concrete surfaces. No significant difference ($P > 0.05$) was found when comparing groups A and B suggesting that the product has poor residual effect for *L. monocytogenes* and *L.* Scott A. Significant reductions ($P < 0.05$) of *L. innocua* populations were observed when groups A and B were compared, indicating a possible residual effect for this strain.

Significance: Biosealed for concrete is a potent antimicrobial and has the potential to be used in combination with other GMPs and other sanitation practices to control bacterial colonization on concrete surfaces.

P3-68 Safety and Shelf Life of Modified Atmosphere-packed and Vacuum-packed Chilled Food Products with Respect to Risks of Psychrotrophic *Clostridium botulinum*

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Introduction: In the UK, the shelf life of vacuum-packaged and modified atmosphere-packaged chilled foods is limited to 10 days unless the product meets at least one of the recommended controlling factors ($a_w \leq 0.97$; pH ≤ 5.0; aqueous salt level > 3.5%; heat treatment 90°C /10 min or equivalent). There are no recommendations for levels of any other preservative factor, or for the use of salts other than sodium chloride. These recommendations are based on controlling the risk from growth of pychrotrophic strains of *Clostridium botulinum* if present in these types of products.

Purpose: To provide manufacturers with a greater range of preservative options when formulating a chilled MAP/VP product.

Methods: Many food products contain preservative factors that are known to have antimicrobial effects as well. This study evaluated the antibotulinal effect of four alternative preservatives (sodium benzoate, potassium sorbate, potassium

lactate and nitrite), either alone or in combination with reduced pH (5.5) and added salts (sodium chloride and potassium chloride). Studies were done in a broth system and a cooked sliced chicken product. Two types of *C. botulinum* cocktail were used, one containing non-proteolytic type B and one containing type E.

Results: Results showed that in the broth system, reduction of the pH to 5.5 had the largest antibotulinal effect at either 8° or 30°C. Additional effects were observed by addition of any of the four preservative types, particularly at 30°C, where pH reduction alone was not sufficient to inhibit growth. In the sliced cooked chicken product, the presence of any of the four preservatives inhibited the growth of *C. botulinum* over a four-week period, in contrast to growth in the control chicken, in which growth of this organism occurred within 3 weeks. Furthermore, the levels of *C. botulinum* type E declined in products containing nitrite and lactate.

Significance: This study has demonstrated the potential for use of benzoate, lactate, sorbate and nitrite as antibotulinal agents in chilled VP/MAP foods.

P3-69 Bacteriophages to Control Foodborne Pathogens in Ready-to-Eat Meat

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Introduction: Bacteriophages have potential for the control of foodborne pathogens. It was shown previously that bacteriophage can reduce the number of many pathogens, either in laboratory media or in foods.

Purpose: Therefore, our objective is to develop systems based on host-specific bacteriophage to enhance food safety by capturing and controlling some important foodborne pathogens in food matrices.

Methods: A cocktail of three lytic bacteriophages active against each of *E. coli* O157:H7, *Salmonella* Enteritidis, *Listeria monocytogenes* and *Shigella boydii* were tested in TSB at 4 and 25°C. The Bioscreen C was used to determine the most effective multiplicity of infection (MOI) for each cocktail to control its respective host over a five day incubation period at 25°C. *Listeria* phages have been tested for their ability to control *Listeria monocytogenes* in Ready-to-Eat oven roasted turkey under different packaging (aerobic, anaerobic and MAP) conditions and temperatures (4° and 25°C).

Results: Results showed that cocktails of phage were able to reduce bacterial growth by 1 to 2.6 log cycles at 4°C, while at 25°C reductions were from 2 to 7 logs. For the *Salmonella* at 25°C, the difference between control and treated samples was not noticeable. The Bioscreen results showed that a MOI higher than 0.1 was sufficient to inhibit growth of *Listeria monocytogenes*, while MOI of around 10^3 to 10^5 was required to cause complete growth inhibition for the other target pathogens. Furthermore, the *Listeria* phage cocktail led to reductions in count of *Listeria monocytogenes* of between 1 to 2.2 log cycles in the meat at different storage conditions.

Significance: These findings suggest that using a cocktail of lytic bacteriophages is a useful biocontrol option that can be used alone or in combination with other preservation techniques to enhance food safety.

P3-70 Control and Prevention of *Cronobacter sakazakii* and *S. enterica* Typhimurium by Each Virulent Phage in Powdered Infant Formula

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Introduction: Cronobacter sakazakii (*Enterobacter sakazakii*) and *S. enterica* Typhimurium are major foodborne pathogens in powdered infant formula (PIF). Especially, these pathogens can lead to serious developmental effects and death in infants.

Purpose: The aims of this study were reduction of *C. sakazakii* and *S. enterica* Typhimurium by using virulent phages in PIF.

Methods: Phages were isolated from swine feces and analyzed plaque assay, transmission electron microscopy, DNA restriction enzyme patterns and structure protein profiles.

Results: As the results of screening, seven *C. sakazakii* phages (CS) and five *S. enterica* Typhimurium (ST) phages were newly isolated. Bacterial cell lytic activity was dependent on infection temperature and concentration of each phage. Each virulent phage was selected for further testing for infection efficiency. CS2 and ST2 phage effectively prevented growth of target pathogens in culture broth and powdered infant formula at various temperatures.

Significance: In conclusion, we think that use of the virulent phages might be reduce the risk of *C. sakazakii* and *S. enterica* Typhimurium infection in PIF.

P3-71 Isolation and Identification of Bacteriophages against *Salmonella* Typhimurium

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Introduction: Salmonella is one of the most common causes of enteric foodborne infections. Among all *Salmonella* serovars, *S.* Typhimurium is responsible for the majority of human cases.

Purpose: The goal of this project was to develop a collection of bacteriophages isolated from manure specific against *Salmonella*. Specific objectives included isolation, identification, characterization and evaluation of phages for controlling *Salmonella* Typhimurium.

Methods: Animal waste samples were collected from a dairy cattle herd and a swine barn. The bacteriophage isolation method included enrichment, spot test, filtration, plaque assays, purification and amplification. *Salmonella* Typhimurium strains were used as host strains in the isolation process. Isolated phages were tested against pure cultures of different *Salmonella* strains including Typhimurium, Newport, Montevideo, Agona, Enteritidis and Heidelberg serovars. The efficiency of plaquing (EOP) method was used to measure lytic activity of phages. Some phages were tested against *Escherichia coli* O157:H7 strains to evaluate the broad spectrum activity. Transmission electron microscopy (TEM) was used to observe phage morphology.

Results: From twenty-four manure samples, a total of nineteen extracts had some lytic activity against *Salmonella* and ten phages were further purified. Those phages were capable of lysing three different *Salmonella* Typhimurium strains, including one multidrug resistant isolate with high EOP values (range: 0.05 to 1.0). None of the phages were active against four *Salmonella* Newport, one *S.* Montevideo, and two *S.* Enteritidis. None of them were effective to lyse *E. coli* O157:H7 strains. Phages were lytic against one *S.* Heidelberg. Phage morphology indicated that they belonged to Group 1 with double stranded DNA, Order Caudovirales and Family Siphoviridae.

Significance: The phages showed very specific lytic activity against *Salmonella* Typhimurium strains. As a natural pathogen of bacteria, when applied to foods, phages might contribute to reducing the incidence of foodborne *Salmonella* as well as the multidrug-resistant isolates.

P3-72 Effect of Contact Time, Dose, Storage Time and Temperature on the Efficacy of Bacteriophage Listex P100 in Reducing *Listeria monocytogenes* Counts on the Surface of Fresh Catfish Fillet Tissue

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Introduction: Listex P100 (phage P100) is a bacteriophage preparation approved by FDA and USDA for raw and Ready-to-Eat foods. Currently, there is no proof of concept on the effectiveness of this lytic bacteriophage in killing *Listeria monocytogenes* on fresh catfish fillet tissue.

Purpose: The objective of this project is to determine the effectiveness of bacteriophage Listex P100 in reducing *Listeria monocytogenes* on fresh catfish fillet tissue as a function of: (i) Listex P100 contact time; (ii) Listex P100 dose; (iii) storage temperature; and (iv) storage duration.

Methods: Fresh catfish fillet tissue was surface inoculated with ~4.3 log CFU/g of two serotype mixture (1/2 and 4b) of *L. monocytogenes* cells and then surface treated with phage P100 by the spot method at different doses and contact times. After specified incubation periods ranging up to 15 min, 30 min, 1 h, 2 h, 1 day, 4 day, 7 day or 10 days, the fillet tissue pieces were subjected to *L. monocytogenes* enumeration.

Results: Phage P100 efficacy was influenced by contact time and application dose regardless of higher or lower temperature regimes tested on catfish fillet tissue. Phage P100 dose of 7.7 log PFU/cm^2 yielded a significant reduction of 1.5 to 1.7 log CFU/g in *Listeria monocytogenes* load on the surface of catfish fillet tissue at all three temperatures regimes tested (4°C, 10°C and room temperature). A contact time of 30 min with phage P100 treatment was essential to yield such reductions in *Listeria monocytogenes* counts on catfish fillet tissue. Such overall reductions in *L. monocytogenes* counts were still maintained at the end of the 10-day shelf life of fresh catfish fillet tissue stored at 4°C or 10°C after phage P100 treatment.

Significance: Our findings illustrate the effectiveness of lytic bacteriophage Listex P100 in reducing *Listeria monocytogenes* load from the surface of fresh catfish fillet tissue. This is the first proof of concept on the listericidal activity of FDA-approved/USDA-approved bacteriophage Listex P100 on the surface of fresh channel catfish fillet tissue.

P3-73 Destruction of High and Low Inoculum Concentrations of *Listeria monocytogenes* on the Surface of Raw Salmon Fillet Tissue by Bacteriophage Listex P100

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Introduction: Ensuring the safety of seafood products from contamination with *Listeria monocytogenes* is a continuing challenge. New classes of GRAS (generally recognized as safe) antimicrobial agents are in strong demand to control the prevalence of foodborne pathogenic bacteria. Among these, host specific lytic bacteriophages are candidates of continuing interest for food safety applications.

Purpose: There is a high prevalence of *L. monocytogenes* in raw salmon fillets. The objective of this study is to determine the effect of FDA-/USDA-approved lytic bacteriophage Listex P100 (phage P100) against low and high inoculum concentrations of *L. monocytogenes* on the surface of raw salmon fillet tissue.

Methods: Raw salmon fillet tissue was surface inoculated with two serotype mixture (1/2 and 4b) of *L. monocytogenes* cells at 1, 2, 3 and 4 log CFU/g and then surface treated with phage P100 at a dose of 7.7 log PFU/cm^2 by the spot method. After incubation for 30 min or 2 h at room temperature, the fillet tissue pieces were tested for surviving *L. monocytogenes*.

Results: There were significant reductions in *L. monocytogenes* counts on fresh salmon fillets tissue at all inoculum loads when treated with phage P100 at a dose of 7.7 log PFU/cm^2 compared to no phage control. Overall, *L. monocytogenes* numbers decreased by 0.9 to 2.3 log CFU/g within 30-min to 2-h treatment with phage P100 depending on the initial inoculum load. For example, *L. monocytogenes* counts were decreased to 0.1 from 1 log CFU/g, or to 0.2 to 0.3 from 2 log CFU/g, or to 0.7 to 1.5 from 3 log CFU/g, or to 2 to 2.5 from 4 log CFU/g on fillet tissue.

Significance: Bacteriophage Listex P100 was found to be highly effective in reducing *L. monocytogenes* counts on the surface of fresh salmon fillet tissue. The antilisteria activity of bacteriophage P100 was confirmed at both low and high inoculum levels commonly occurring on raw salmon fillet tissue.

P3-74 Characterization of Lytic Bacteriophages against *Bacillus cereus* for Potential Use as Bio-control Agents DSC

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Introduction: Bacillus cereus is a foodborne pathogen associated with foods prepared for large groups of people. As a spore-forming bacterium, *B. cereus* is resistant to several processing steps, especially those that are dependent on heat application. Lytic bacteriophages have received increasing attention as potential agents for the control of pathogenic bacteria.

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Purpose: This study aimed to isolate and characterize bacteriophages specific for *B. cereus* and able to cause complete lysis of the target organism.

Methods: Sewage and sludge samples were collected and mixed with a cocktail of *B. cereus* strains to test for the presence of bacteriophages. Isolation of bacteriophages was based on the plaque size and shape. Transmission electron microscopy was carried out to examine phage morphology. Different strains of *B. cereus* as well as other *Bacillus* species were used to determine the host specificity of the isolated phages. The sizes and restriction patterns of the phage DNA was determined. The infectivity rate was tested under the stress of some chemical factors such as chloroform, and physical factors such as ambient and high temperatures.

Results: Four bacteriophages were isolated and purified. TEM images showed three phages with isometric heads and long non-contractile tails, believed to belong to the family Siphoviridae. A fourth phage with an isometric head and contractile tail was provisionally identified as belonging to the Myoviridae. The isolated phages showed different degrees of infectivity and specificity against tested organisms. Different restriction enzymes showed distinct restriction DNA patterns for the isolated phages. Also the phages showed different responses depending on the level of the stress of chemical and physical factors.

Significance: This study recommends using the isolated phages to control *B. cereus* and suggests that the effect will be maximized if combined with other preservation methods.

P3-75 Isolation and Characterization of Lytic Bacteriophages against Enterohemorrhagic *Escherichia coli*

DSC

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Introduction: Since the 1980s, Enterohemorrhagic *Escherichia coli* (EHEC) strains have been recognized as foodborne pathogens and serotype O157:H7 has caused frequent gastroenteritis outbreaks. Bacteriophages can be used as a natural non-antibiotic method with the potential to reduce bacterial pathogens, such as EHEC, from the food supply.

Purpose: The objective of this study was to isolate, identify and characterize a diverse collection of lytic bacteriophages capable of infecting EHEC serotypes O26, O111 and O157.

Methods: Phages previously isolated were provided by Dr. Andrew Brabban and Dr. Lawrence Goodridge. Additional phages were isolated from dairy and feedlot manure using EHEC O157, O26 and O111 strains as hosts. Manure was enriched using Tryptic Soy (TS) Broth and exponentially growing cultures of specific bacterial hosts, and was incubated overnight at 37°C. The enriched extracts were centrifuged, filtered, combined with the host strain in tryptone top agar, and plated on TS agar. Plaques were purified and screened against additional strains (14, O157; 10, O26; 10, O111) using the efficiency of plaquing method (EOP). Purified phages were observed using transmission electron microscopy (TEM) after staining with 2% ammonium molybdate.

Results: Phage CEV2 and five other phages previously isolated were able to lyse all 14 O157 strains with EOP values consistently above 0.001. Four out of six phages isolated from a cattle feedlot were effective against all O157 strains and one O26 strain with EOP values greater than 0.001. After spot testing, they were found to be effective against seven O26 strains. Five phages isolated from dairy manure were highly specific against O26 strains but were not effective against other serotypes. Based on TEM, some of the phages were classified as Group 1 Caudovirales.

Significance: These results indicated that the isolated bacteriophages were highly effective against multiple strains of two EHEC serotypes. This collection of phages can be grouped and potentially used as an antimicrobial cocktail to inactivate O157 and O26 serotypes and reduce their incidence in the food chain.

P3-76 Analysis of Antimicrobial Resistance in *Enterococcus* spp. Recovered from a Commercial Beef Processing Plant

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Introduction: Antimicrobial resistant enterococci are considered to be a human health concern, as they can provide a pool of resistance genes transferable to pathogens.

Purpose: The goal of this study was to analyze antimicrobial- resistant (AMR) phenotypes and resistance genes in enterococci isolates from a commercial beef processing plant.

Methods: Samples were obtained from conveyers used for moving carcasses, before the start of operation (CC), two hours after operation has started (DC), and from ground beef (GB). Samples were screened for presumptive enterococci on KF agar, and three isolates from each positive sample (26 from CC; 27 from DC; 26 from GB) were randomly selected and confirmed to species level using PCR and the API® 20 Strep kit. A total of 201 isolates comprising 48, 81 and 72 from CC, DC, and GB, respectively, were analyzed for AMR and resistance genes and were genetically characterized. The AMR was determined by use of a sensititre system®, and results were interpreted according to the criteria of the Clinical Laboratory Standard Institute. PCR was used for the detection of resistance genes.

Results: Enterococcus faecalis (80%) was the most common species found, followed by *E. faecium* (6%) and *E. durans* (3%). Complete resistance to tetracycline and lincomycin was found in 48% and 10% of isolates, respectively, and 83%, 59% and 37% of isolates were intermediate resistant to lincomycin, erythromycin and ciprofloxacin, respectively. Resistance to quinupristin/dalfopristin was found in 36% of *E. faecium* isolates. Tetracycline resistant enterococci were positive for *tetM* gene, whereas *ermB* and *vatE* genes were found in 18% and 49% of enterococci. The analysis showed that the majority of enterococci from individual sample sources were genetically related. Data on the prevalence of antimicrobial resistant enterococci and resistant genes among various sample sources will be discussed.

Significance: These findings suggest that antimicrobial resistant *Enterococcus spp.* are prevalent during commercial beef processing. This study underscores the need for improving hygienic conditions in meat processing plants to improve the safety of meat.

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P3-77 Antimicrobial Susceptibility of *Arcobacter butzleri* Isolated from Korean Chickens

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Introduction: An arcobacter species was originally known as the causative pathogen related to abortion of bovine and porcine fetuses. Recently, this has become one of the newly emerging foodborne pathogens that causes persistent diarrhea, abdominal pain, nausea, and vomiting in humans.

Purpose: The aim of this study was to investigate the antimicrobial susceptibility of *Arcobacter butzleri* isolated from chickens in Korea.

Methods: Thirty-nine *A. butzleri* were isolated from chickens in Korea and confirmed by multiplex polymerase chain reaction. Disk diffusion susceptibility test against 22 antimicrobial agents was performed by the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) M31-A2.

Results: All isolates were susceptible to amikacin, gentamicin, tobramycin, and linsmycin (lincomycin + spectinomycin). Ninety-five percent of *A. butzleri* isolates were susceptible to kanamycin, streptomycin, and neomycin. Chloramphenicol, cefuroxime, erythromycin, and lincomycin were associated with moderate susceptibility in more than 50% of *A. butzleri* isolates. In contrast, all isolates showed resistance to cephalothin, penicillin, sulfamethoxazole, and vancomycin. More than 50% of isolates were resistant to enrofloxacin, ciprofloxacin, tiamulin, tylosin, and ampicillin.

Significance: Amikacin, gentamicin, tobramycin, linsmycin were the most effective antibiotics in suppressing and controling growth of *A. butzleri* isolates. However, antimicrobial resistance against multiple drugs was found in *A. butzleri* Korean isolates.

P3-78 Resistance of *Pediococcus pentosaceus* to Antibiotics Used in Food Animals
DSC

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Introduction: Lactic acid bacteria (LAB) have long been used in the production of fermented foods. It is possible that among LAB used as starter cultures and probiotics, those which demonstrate antibiotic resistance may serve as reservoirs of resistance genes transferable to pathogens.

Purpose: The objective of this study was to determine the level of resistance of meat starter cultures to various antimicrobial agents registered in Canada for use in food animals.

Methods: Microbiological breakpoints (concentrations defining resistance) of ten strains of *Pediococcus pentosaceus* to 20 antimicrobial agents were investigated using the microdilution method with a mixed formulation of Iso-Sensitest broth (90% v/v) and deMan-Rogosa-Sharpe broth (10% v/v). Minimum inhibitory concentrations were determined according to the breakpoint values established by the European Food Safety Authority (EFSA). For those antibiotics which have no established microbiological breakpoint, the breakpoint value was derived from clinical performance of the antimicrobial class as reported in the literature.

Results: Results showed that 100% of the *P. pentosaceus* strains were resistant to tetracycline and oxytetracycline. For antibiotics belonging to the macrolides class, 40% of the strains showed resistance. Aminoglycoside and penicillin antibiotics were uniformly effective against the *P. pentosaceus* strains.

Significance: These results show that among *Pediococcus* strains used as meat starter cultures, there can be a significant reservoir of antibiotic resistance capability. Further work will characterize the ease with which this resistance may be transferable to organisms of public health concern in food.

P3-79 Antibiotic-resistant Enterobacteriaceae Isolates from Retail Meats and Domestic Kitchen Environment in Tennessee

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Introduction: Contamination of food by antibiotic-resistant bacteria raises immediate concerns for public health. It is essential to determine the sources of antibiotic-resistant bacteria, whether from the environment or food products.

Purpose: The purpose of this study was to identify antibiotic-resistant profiles of Enterobacteriaceae isolates from raw meats and domestic kitchens in Tennessee.

Methods: Retail meats (n = 357) and domestic kitchen (n = 443) samples were collected and analyzed between summer 2007 and spring 2008. Meat (beef, chicken, turkey, pork, guinea fowls) were purchased from local stores. Various locations in domestic kitchens were swabbed with sterile and moistened sponges. Butterfields phosphate buffer (25 ml) was added to each meat sample (25 g), each sponge sample, and pummeled in a Stomacher 400 Circulator at 230 rpm for 2 min. The homogenate was then used for further analysis. Biochemical methods were used for characterization and identification of bacteria. Antibiotic resistance against selected antibiotic was determined by Kirby-Bauer technique.

Results: Retail meats were contaminated with a range of bacterial pathogens including, *Klebsiella* (23.8%), *Escherichia coli* (10.65 %), *Salmonella* spp (6.16%), *Morganella morganii* (0.84%), and *Yersinia enterocolitica* (0.28%). *Enterobacter sakazakii* (6.55%) and *Klebsiella* (16.67%) were recovered from domestic kitchen environments. *Salmonella* and *Escherichia coli* were further identified as *Salmonella arizonae*, *Salmonella pullorum*, *Salmonella gallinarum*, *Salmonella choleraesuis*, *Escherichia coli* O157:H7, and *Escherichia coli 1*. *Klebsiella* spp was also indentified as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella terrigena*. The isolates showed single, double, and triple antibiotic resistance. Resistance was recorded for tetracycline (30.27%), ampicillin (52.29%), streptomycin (52.3%), kanamycin (22.01%, erythromycin (100%), penicillin (90.8%), chloramphenicol (4.6), and oxytetracycline (22.01%).

Significance: Retail meats and domestic kitchen environments can be sources of pathogenic antibiotic-resistant bacteria.

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P3-80 Antimicrobial Resistance in *Escherichia coli* O157:H7 from Patients in Alberta

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Introduction: The use of antibiotics in animals and agriculture may select for antimicrobial resistance (AMR) in *Escherichia coli* O157:H7. Consumption of AMR bacteria can lead to colonization of the human gastrointestinal tract. Although *E. coli* O157:H7 infections are not clinically treated with antibiotics, horizontal transfer of resistance genes to susceptible commensal flora can occur and can have serious implications.

Purpose: This study was undertaken to determine trends in AMR and assesses strain clonality through phage typing (PT) and pulsed-field gel electrophoresis (PFGE) among *E. coli* O157:H7 isolates associated with human disease.

Methods: E. coli O157:H7 isolates (n = 430) from human origin were isolated by the Alberta Provincial Laboratory for Public Health (APLPH). Sporadic cases and single representative isolates from each outbreak in 2003 to 2006 were subtyped by PFGE at APLPH and PT at the Canadian National Microbiology Laboratory. AMR profiling was obtained using microbroth dilution testing with 15 antimicrobial agents.

Results: Of the 430 *E. coli* O157:H7 isolates, 39 (9.1%) were resistant to at least one antibiotic and 26 (6.0%) were resistant to two or more antibiotics, while the most frequent resistance was to sulfisoxazole (5.3%), tetracycline (4.9%), and chloramphenicol (3.3%). All of the AMR *E. coli* O157:H7 strains typed as having unique PT and PFGE patterns. An increase in nalidixic acid resistance was observed from 1.0% in 2004 to 3.4% in 2006, and these isolates were limited to two PTs.

Significance: Overall AMR among *E. coli* O157:H7 strains associated with human disease collected between 2003 and 2006 was not common and resistant strains demonstrated no clonality by PT or PFGE. This suggests that the risk of transferring AMR to commensal gut flora was low; however, multi-drug resistance and resistance to nalidixic acid was observed, suggesting that emerging AMR trends should be monitored over time.

P3-81 Pediocin PA-1-like Bacteriocin Produced by *Enterococcus faecium* ST5HA

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Introduction: Bacteriocins of lactic acid bacteria (LAB) are ribosomally synthesized antimicrobial peptides. Their bactericidal mechanisms may include pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rDNA, and inhibition of peptidoglycan synthesis.

Purpose: This research is on isolation of an anti-*Listeria* bacteriocin producing strain, characterization of the genetic determinants of bacteriocin production, and study of some aspects of the bacteriocin mode of action.

Methods: The bacteriocin-producing strain ST5HA was isolated from smoked salmon and identified on the basis of API50CHL, PCR with genus-specific-primers and sequencing of the 16s rDNA. The homology of the produced bacteriocin ST5HA to pediocin PA-1 was determined with PCR and sequencing of the amplified product.

Results: Enterococcus faecium ST5HA produces a pediocin-like bacteriocin with activity against several LAB, *Listeria* spp., and some other human and food pathogens. Bacteriocin ST5HA was produced at high levels in MRS broth at 30°C and 37°C and reached maximum production (1.0×10^9 AU/ml against *Listeria ivanovii* subsp. *ivanovii* ATCC19119) after 43 h. Its molecular mass, 4.5kDa, was determined by tricine-SDS-PAGE. Addition of bacteriocin ST5HA to a 3-h-old culture of *L. ivanovii* subsp. *ivanovii* ATCC19119 inhibited growth for 24 h. Strain ST5HA contains a 1044 bp DNA fragment corresponding in size to that recorded for pediocin PA-1. The combined application of low levels (below MIC) of ciprofloxacin and bacteriocin ST5HA results in a synergetic effect in the inhibition of *L. ivanovii* subsp. *ivanovii* ATCC19119.

Significance: To our knowledge, this is first report on *E. faecium* ST5HA as a potential producer of the chromosomally associated pediocin PA-1-like bacteriocin, based on the high similarity to the sequence of pedB. Bacteriocin ST5HA is highly active against *L. ivanovii* subsp. *ivanovii* ATCC19119 and exhibits a synergetic effect in the inhibition of this test microorganism when applied with sublethal doses of ciprofloxacin.

P3-82 DSC Effect of *Carnobacterium maltaromaticum* UAL 307 and *Enteroccocus faecalis* 710C Cultures and Culture Supernatants on the Growth of *Listeria monocytogenes* in Fresh Beef Sausage

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Introduction: Bacteriocins are inhibitory compounds produced by several bacteria, including *Carnobacterium maltaromaticum* and *Enterococcus faecalis* and inhibit the growth of *Listeria monocytogenes* in meat products. Recent studies have shown the potential of *C. maltaromaticum* to enhance the color stability of fresh meat.

Purpose: The purpose of this study was to use bacteriocin-producing lactic acid bacteria and their culture supernatant to inhibit the growth of *L. monocytogenes* in fresh beef sausage and determine the effect of *C. maltaromaticum* on sausage color stability.

Methods: Lean ground beef was used to make fresh sausage in collagen casings, which were inoculated (except for a control treatment) with a cocktail of *L. monocytogenes* and *C. maltaromaticum* UAL 307 at approximately 10^4 CFU/g and 10^5 CFU/g, respectively, combined with the supernatants of *C. maltaromaticum* UAL 307 or *E. faecalis* 710C. Sausages were packaged either aerobically or in an atmosphere of 30% CO_2 + 0.4% CO and stored at 4°C for up to 10 or 15 days. The growth of *L. monocytogenes* and sausage pH and color were measured either every two days (aerobic storage) or every three days (anaerobic storage).

Results: Sausages containing *C. maltaromaticum* UAL 307 with either of the supernatants eliminated *L. monocytogenes* after 8 days of aerobic storage and 12 days of anaerobic storage; addition of *C. maltaromaticum* UAL 307 alone resulted

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in a greater than 2 log reduction in numbers of *L. monocytogenes* after 6 or 12 days in aerobic and anaerobic storage, respectively. The addition of cultures had no effect on pH or color of anaerobically storage sausages; sausages stored aerobically with *C. maltaromaticum* UAL 307 maintained a red color during 10 days of storage.

Significance: Results indicate that *C. maltaromaticum* UAL 307 inhibits the growth of *L. monocytogenes* in fresh beef sausage, and eliminates *L. monocytogenes* cells when combined with supernatant. Combined with color stabilizing properties and negligible effect on meat pH, *C. maltaromaticum* UAL 307 provides the meat industry with an effective intervention that would extend the retail storage life of fresh sausage.

P3-83 DSC Effect of Antimicrobial Packaging on Control of Spoilage Microorganisms on Naturally Contaminated Ready-to-Eat Meats

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Introduction: Active packaging has been used to improve the shelf life of food by inhibiting the growth of spoilage microorganisms. Our previous studies have shown that packaging structures containing a CO_2 generator or O_2 scavenger significantly inhibited the growth of *Listeria monocytogenes* on artificially contaminated Ready-to-Eat meats.

Purpose: The purpose of this study was to evaluate the efficacy of the same packaging structures on control of spoilage microorganisms on four naturally contaminated Ready-to-Eat meat products.

Methods: Cooked ham, roast beef, roast chicken and roast turkey meats were purchased from a local grocery store. Each meat product (25 g) was placed in the packaging structures containing a CO_2 generator, a O_2 scavenger, or both the CO_2 generator and O_2 scavenger. Traditional, non antimicrobial structure were included in the study as controls. The packaged meats were stored at 4, 10, and 22°C for 3 weeks. The meats were sampled weekly for the populations of aerobic bacteria, lactic acid bacteria, and *Pseudomonas* as well as yeast and mold.

Results: Meats packaged in the three antimicrobial packaging structures had significantly lower microbial populations in comparison to those in the non antimicrobial packaging structure. The packaging structures with the CO_2 generator or the O_2 scavenger reduced the total aerobic bacteria, yeast and mold, *Pseudomonas* and lactic acid bacteria by 1.83 to 2.13, 1.41 to 2.70, 1.03 to 1.61, and 0.03 to 0.67 log CFU/g, respectively. The packaging structures with both the CO_2 generator and O_2 scavenger reduced the populations of aerobic bacteria by 2.64 to 4.07 log CFU/g. The average yeast and mold counts were reduced by 2.74 to 3.36 log CFU/g. The lactic acid bacterial were reduced by less 1 log CFU/g on all meats except roast beef, while the Pseudomonas populations were reduced by 1.61 to 2.58 log CFU/g.

Significance: The study suggests that the CO_2 generator and O_2 scavenger were effective in inhibiting the growth of sampled microorganisms on Ready-to-Eat meat products.

P3-84 A Science-based Approach to Calculating Safe Cooking Temperatures for Poultry Meat in New Zealand

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Introduction: Historical New Zealand guidelines for core cooking temperatures for whole poultry meat varied from 72°C to 82°C. For clarification, the New Zealand Food Safety Authority published an interim recommendation for core cooking temperatures at 82°C until a robust science-based time/temperature combination could be validated. As expected, both industry and consumers regarded this high temperature as excessive, resulting in poultry products of poor quality.

Purpose: To provide a scientifically-derived range of temperature/time combinations that achieve a 6 log CFU/g thermal reduction of *Listeria* spp., *Salmonella* and *Campylobacter* spp. in poultry meat.

Methods: A literature review was undertaken to locate relevant publications. Data from the literature were entered into a Microsoft® Access® database along with available contextual information affecting D-values: strain and origin of the pathogen, phase of growth, heating method, composition of the heating menstruum, etc. Only data from studies using poultry meat were included. Data displaying non-linear shoulder or tailing effects were excluded.

Results: Over 300 relevant D-values for poultry meat were identified. Based on linear regression plots of D-time versus temperature, *Listeria* spp. were clearly the most heat resistant pathogen. Achieving a 6 log CFU/g reduction in *Listeria* populations would therefore result in greater reductions in *Salmonella* and *Campylobacter* numbers. D-values for *Listeria* spp. were adopted using the 95% percentile of the data at each temperature. Cooking recommendations ranged from achieving 60°C for 10 min to instantaneous destruction at 75°C.

Significance: A comprehensive analysis of available data was able to offered science-based answers to a specific food safety question. The development of experimental protocols to directly address such questions and produce additional data points in order to understand the highly variable nature of the data would also be valuable.

P3-85 DSC Selecting Susceptible and Resistant *Salmonella* Serovars for Cocktail Preparation: A Case Study in Mitigating Sample Bias Using Statistical Methods

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Introduction: Sample bias is a mathematical property that reflects the difficulty in obtaining a truly representative sample from a population. In microbiological fields, bacterial strains are often chosen intuitively for research, sometimes deliberately with the intent of shaping the results, with no well-defined formal criteria. This can lead to unpredictable and/or misleading results.

Purpose: The objective was to prepare two bacterial cocktails comprised of *Salmonella* serovars by selecting them from a bank of isolates, such that sample bias was mitigated.

Methods: The sample population consisted of 52 naturally occurring *Salmonella* isolates of various serovars, 23 of which were antibiotic susceptible *Salmonella*, and 29 that were ACSSuT-resistant. A disk diffusion susceptibility test was conducted on both PCA and XLT4 agars, using discs soaked in a 3% lactic acid solution. The diameter of the zones

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of inhibition (ZOI) produced were measured. Furthermore, a five-isolate cocktail was required for both resistant and susceptible *Salmonella*. The ZOIs for each isolate were paired together, and median ZOIs were calculated separately for each *Salmonella* type. The Euclidean distances were calculated and normalized, and outliers were removed if their normalized distance was significantly higher than most other data points. Verification of outlier removal was also visually observed with scatter plots. The two data sets were partitioned, using hierarchical clustering with Euclidean distances. The results were plotted as dendrograms, and the specific isolates were then chosen.

Results: For the case study, significant differences ($P < 0.05$) were observed in the diameter zones of inhibition for ACSSuT-resistant and susceptible *Salmonella* on both PCA ($P = 0.025$) and XLT4 ($P = 0.046$). The sample error for all resistant and susceptible Salmonella samples is 0.198 and 0.237, respectively. This high error rate is due to limited sample availability, illustrating the importance of a large sample size. Five isolates were then chosen for each cocktail based on their ZOI similarity in the dendrograms, because the behavior of these isolates are more likely to represent the behavior of serovars in the general *Salmonella* population.

Significance: When statistical methods, such as the one discussed here, are applied to a study, the results are more valid. If all researchers were to embrace these types of sample selection methodologies, there would be fewer false positives and fewer misleading and irreproducible results, and it could even lead to increased perception and trust among professionals and the general public alike.

P3-86 A Response Surface Model to Describe the Effect of Temperature and pH on the Growth of *Bacillus cereus* in Cooked Rice

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Introduction: A predictive model is being popularly applied to food industry to estimate *Bacillus cereus* growth and to determine the shelf life of cooked rice.

Purpose: This study was performed to develop a predictive model for the growth rate of *B. cereus* in cooked rice using a response surface methodology (RSM).

Methods: B. cereus F4810/72 producing an emetic toxin was used in this study. The growth curves as a function of storage temperature (10 to 40°C) and pH value (5.4 to 6.8) were fitted using a modified Gompertz equation and the relationship of the growth rate to the growth curves was modeled using an RSM quadratic polynomial equation. The RSM evaluation for describing the growth rate of *B. cereus* used both bias (Bf) and accuracy factors (Af).

Results: The predicted growth of *B. cereus* in cooked rice varied from 0.01 h-1 at 10°C to 1.08 h-1 at 40°C. At the lower pH of 5.4, the growth of *B. cereus* was hardly observed regardless of storage temperature. The response surface quadratic polynomial equation was "Y = 0.330861 + 0.313648 × 1 + 0.212498 × 2 + 0.196629 × 1 × 2 + 0.037367 × 12 - 0.032263 × 22". The predictive model was significant ($P < 0.01$) and the predicted values of the growth parameters obtained using the model equations were in good agreement with experimental values (R^2 value of 0.9864). Both the Bf value (1.006) and the Af value (1.011) approached 1.0 and were within acceptable ranges. Therefore, the adequacy of the predictive model for *B. cereus* in cooked rice was verified by the validation data.

Significance: Our model can be used as a reference in controlling *B. cereus* growth in cooked rice without the need for detection of the organism and may be of use to manufacturers of rice products for controlling *B. cereus* growth.

P3-87 Growth of *Escherichia albertii* on Ground Beef Stored at Various Temperatures
DSC

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Introduction: Escherichia albertii is the newest species within the genus *Escherichia* and may represent an emergent foodborne pathogen. *E. albertii* has been cultured from stool samples of diarrheagenic children repeatedly and some strains are capable of synthesizing the pathogenesis factor intimin. The influence of food storage temperature on growth and survival of *E. albertii* in various foods has not been widely examined.

Purpose: The objective of this study was to determine the growth characteristics of *E. albertii* on fresh raw ground beef as a function of storage temperature.

Methods: Nalidixic acid-resistant strains of *E. albertii* (American Type Culture Collection 19982, 10457, and 9194) were individually inoculated on 150 g samples of ground beef to a concentration of 4.3 ± 0.7 log CFU/g. Aliquots (11 g) were prepared and incubated aerobically at 5°C, 22°C, or 35°C for 21, 7, or 3 days, respectively. *E. albertii* were enumerated following spread plating on MacConkey Agar supplemented with L-+-Rhamnose (10 g/l) and antibiotic (50 mg/l). The experiment was replicated three times. Means from like samples were generated and statistically significant differences assessed as a function of pathogen strain, days of incubation, and temperature of incubation ($P < 0.05$).

Results: E. albertii 19982, 10457, and 9194 populations decreased by approximately 2.0, 1.6, and 0.9 log cycles following 21 days of storage at 5°C, respectively. Populations of *E. albertii* 19982, 10457, and 9194 recovered from beef incubated at 22°C and 35°C following 7 and 3 days of incubation, respectively, increased by 2.5, 3.6, and 3.7 log cycles and 3.9, 3.5, and 3.9 log cycles.

Significance: E. albertii is able to flourish on nutrient-rich foods under conditions of abuse or at physiological temperature. However, data indicate that proper refrigeration of food products can assist in the prevention of growth of this potential foodborne pathogen. Further research on the ability of *E. albertii* strains to synthesize intimin along with its growth and survival on various other foods should be conducted.

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P3-88 Cold Tolerance of *Clostridium perfringens* Induced by GRAS Substances

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Introduction: C. perfringens is an anaerobic, spore-forming foodborne pathogen that occurs in a variety of foods. Symptoms associated with poisoning are caused by *Clostridium perfringens* enterotoxin (CPE) that is expressed during sporulation of enterotoxigenic strains (Heredia and Labbé 2001). Physical and chemical treatments are used in food processing to eliminate or reduce the presence of pathogens and spoilage microorganisms. Sodium benzoate and potassium sorbate are GRAS (Generally Recognized As Safe) substances and are commonly used as preservatives (USFDA 2003).

Purpose: In this work we investigate the effect of GRAS substances on cold tolerance of strains of *Clostridium perfringens*.

Methods: Maximal concentrations recommended for foods of sodium benzoate, potassium sorbate, sodium nitrite, monosodium glutamate, or mixtures of those were added to cultures, and their effect on *C. perfringens* tolerance to 10°C was evaluated. The effect of a previous shock at 28°C was also determined.

Results: Growth of *C. perfringens* was not inhibited by the substances examined. Sodium nitrite, applied at maximal permitted concentrations, increased *C. perfringens* survival at 10°C. Mixtures of GRAS compounds either had no clear effect or caused an increase of tolerance to 10°C. A pre-shock (28°C) of the cultures treated with sodium benzoate, sodium nitrite or monosodium glutamate increased survival and stimulated growth of the cultures treated at 10°C.

Significance: We conclude that the addition of GRAS substances, including sodium benzoate, potassium sorbate, sodium nitrite, and monosodium glutamate, to cultures of *C. perfringens* can influence their cold tolerance. In some cases, these substances that would normally eliminate microorganisms can increase tolerance, permitting survival at low temperatures. The acquired cold tolerance of *C. perfringens* by these preservatives could represent a significant hazard.

P3-89 Effects of Temperature and pH on the Thermal Inactivation of *Bacillus pumilus, B. licheniformis, B. subtilis* and *B. megaterium*

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Introduction: Bacillus pumilus, *B. licheniformis*, *B. subtilis*, and *B. megaterium* have been traditionally associated with the spoilage of food products; however, more recently there has been an increased concern for the potential food safety implications associated with these species.

Purpose: The purpose of this study was to obtain heat resistance data relevant to food pasteurization regimes and to model the effects of temperatures and pH on the survival of the spores of these four bacilli.

Methods: A surface response design was used to determine the thermal resistance across a range of temperature (93 to 107°C) and pH (3.6 to 6.4). The results were analyzed using Minitab 15 Statistical Software and a predictive model for each of the four *Bacillus* species studied was proposed.

Results: When these sets of data are compared, the results show a consistent decrease in the D value with decrease in pH. For example, the D values for the four species heated at 95°C at a pH of 6 were 4.56, 3.51, 8.60, and 5.70 compared with 1.30, 2.20, 2.39, and 1.02 at pH 4 for *Bacillus pumilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis*, respectively. When the log of D was plotted against temperature and pH, the models were able to predict thermal death of 91, 89, 94 and 94% for *B. pumilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis*, respectively. For *B. pumilus*, a linear relationship was seen for log D versus pH and temperature. For *B. megaterium*, *B. subtilis* and *B. licheniformis*, the pH showed a quadratic effect. An interaction with pH and temperature was seen for *B. megaterium* and *B. subtilis*.

Significance: Readjusting the processing parameters by increasing the temperature or reducing the product pH will allow a reduction in the number of these food spoilage and potential pathogenic organisms surviving in the final product.

P3-90 Time-temperature Profiling Associated with Preparation and Storage of Powdered Infant Formula: Implications for Microbial Safety

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Introduction: Powdered infant formula (PIF) is not sterile and UK recommendations are that powdered infant feeds are reconstituted using boiled water > 70°C (cooled for 30 min). If it is not possible to reconstitute feeds immediately before use, made-up feeds should be refrigerated and transported in a cool bag for < 4 h. Research indicates frequent non-compliance with this advice.

Purpose: This study determines the time-temperature profiles and microbial counts of PIF reconstituted using water at different temperatures and subsequent storage.

Methods: Time-temperature profiles of three volumes of water (1.5 litre, 1litre, 500ml) were determined for 30 min after boiling in domestic kettles(n = 25) using a datalogger (accuracy ± 0.5°C). 260 ml aliquots of the cooled, boiled water was then used to reconstitute PIF in cleaned/sterilized feeding bottles and sampled for Aerobic Colony Counts (ACC) and Enterobacteriaceae. In separate experiments, internal time-temperature profiles of replicate (n = 5) reconstituted PIF feeds stored at ambient temperature and in cool-bags for > 4 h were recorded using miniature dataloggers (accuracy ± 0.5°C). Initial temperatures of feeds ranged from < 5°C, ~20°C and 70°C in 125 ml/260 ml bottles. ACC, Enterobacteriaceae and *Staphylococcus aureus* counts were determined immediately after reconstitution and before potential feeding.

Results: After 30 min of cooling temperatures of 1.5l boiled water were > 70°C. Microbial counts of feeds reconstituted with this water were < 1.0×10^1 ACC and no Enterobacteriaceae were isolated. However, temperatures of 1litre and 500 ml boiled water, after 30 min were below 70°C and resultant ACC counts were up to 3.4×10^3. The temperature of all feeds stored at ambient temperature and in cool-bags for > 4 h was conducive for microbial growth. No Enterobacteriaceae or *S. aureus* were isolated.

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Significance: Cooling larger quantities of boiled water resulted in temperature remaining > 70°C which has an effect on the microbial quality of the product. Microbial counts increased significantly within 4 h when reconstituted feeds are stored at > 5°C. Studies have shown time-temperature abuse by consumers of over 18 h, and this could have a major impact on PIF quality.

P3-91 Pulsed Electric Field Inactivation of *Escherichia coli* O157:H7 and Surrogate Bacteria in Orange Juice

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Introduction: United States FDA juice HACCP rules mandate that orange juice (OJ) processors treat juice for a 5 log reduction of the target pathogen. Thermal pasteurization, however, reduces the sensory characteristics of OJ by removing or altering volatile compounds. Pulsed electric field (PEF) treatment of OJ, an alternative to thermal pasteurization, may preserve these sensory characteristics.

Purpose: This study was performed to determine inactivation kinetics of *E. coli* O157:H7 in OJ by PEF. Another goal was to screen non-pathogenic bacteria with PEF as potential surrogates for O157:H7 for use in biosafety level 1 pilot plant studies.

Methods: E. coli O157:H7 (ATCC 43895) and twenty-six strains of non-pathogenic bacteria were screened for sensitivity to PEF in OJ with a prototype biolevel 2 PEF system. Cultures were inoculated into orange juice (pH 3.85) at 7 log CFU/ml and treated with PEF at 45 and 50°C, and 22 and 20 kV/cm, respectively. Following this study, survival of the selected surrogate bacterium and O157:H7 were compared at temperatures of 45, 50 and 55°C and field strengths ranging from 6 to 32 kV/cm. Injury was determined by plating onto MacConkey agar + 0.3% sodium deoxycholate.

Results: Screening of surrogate bacteria revealed that the inactivation of *E. coli* ATCC 35218 most closely resembled that of *E. coli* O157:H7. Analysis of covariance, to test for homogeneity of survival curves, determined that the bacteria were statistically indistinguishable at 45 and 50°C, although O157:H7 was slightly more sensitive to PEF than surrogate 35218 at 55°C. Treatment conditions of 55°C and 29 kV/cm resulted in 4.10 log CFU/ml and 100% injury of both bacteria.

Significance: These results indicate that non-pathogenic biosafety level 1 ATCC 35218 is a suitable surrogate for *E. coli* O157:H7 in OJ treated by PEF. Future studies will utilize the surrogate bacterium in scaled-up biolevel 1 pilot plant PEF studies.

P3-92 DSC Impact of Pressure Pulsing on Biochemical Changes of *Bacillus amyloliquefaciens* Spore Inactivation through Fourier Transform Infrared Microspectroscopy

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Introduction: Pressure-assisted thermal processing (PATP) is a promising technology for inactivating bacterial spores at modest process temperatures. To enhance microbial lethality, pressure pulsing has been reported as an effective approach. However, very limited mechanistic understanding is available on microbial efficacy of single- and double-pulse pressure treatments.

Purpose: The objective of this study was to identify spore components that likely cause inactivation during single- and double-pulse treatment. An additional objective was to compare biochemical changes associated with PATP spore resistance influenced by sporulating media (TSAYE- and NAYE-10 ppm $MnSO_4$).

Methods: A spore suspension of *Bacillus amyloliquefaciens* TMW 2.479 in deionized water (~1.6×10^8 CFU/ml) was treated at 600 MPa-105°C. Spore samples were withdrawn at various stages of single- and double-pulse treatment. Survivors were enumerated after incubation at 32°C up to 72 h. Treated and untreated samples were analyzed, using ATR-IR microspectroscopy in the mid-infrared region (4000 to 700 cm^{-1}).

Results: Process temperature during single pulse-pressure holding time was maintained at 105.5 ± 0.4°C. On the other hand, process temperature during the 2nd pulse treatment was increased to 112 ± 0.9°C due to heat of compression. Spore crop grown on NAYE showed higher pressure-heat resistance than that on TSAYE. For equivalent pressure holding time, the double-pulse treatment inactivated up to 2.2 log CFU/ml more of spore population more than that of single-pulse treatment. FT-IR analysis showed that PATP treatment (both single- and double-pulse) caused predominant changes in DPA structures and interactions of Ca^{2+} with COO^- form of DPA and/or acidic proteins. However, differences in spectra region between single- and double-pulse treatments of NAYE crop were associated with secondary amide structure (1543 and 1037 cm^{-1}).

Significance: FT-IR microspectroscopy showed great potential to gain insight into spore inactivation during PATP. Improved understanding of PATP spore inactivation mechanisms will further facilitate technology implementation in the food industry.

P3-93 Inactivation of *Escherichia coli* O157:H7 and Nonpathogenic *E. coli* in Strawberry Juice by Pulsed Electric Field, Sodium Benzoate, Potassium Sorbate, and Citric Acid

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Introduction: Current regulations require that juice processors effect a 5 log CFU/ml reduction of a target pathogen prior to distributing products. Whereas thermal pasteurization reduces the sensory characteristics of juice by altering flavor components, pulsed electric field (PEF) treatment may be conducted at lower temperatures, thus preserving sensory characteristics.

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Purpose: This study was performed to determine the inactivation of *E. coli* O157:H7 and a nonpathogenic *E. coli* in strawberry juice by PEF, with or without sodium benzoate, potassium sorbate, and citric acid.

Methods: E. coli O157:H7 (ATCC 43895) and a nonpathogenic *E. coli* (ATCC 35218) were inoculated at 7 log CFU/ml into single strength strawberry juice with or without 750 ppm sodium benzoate, 350 ppm potassium sorbate, and 2.7% citric acid. Juice was treated at 45, 50 and 55°C and 18.6 kV/cm with a prototype PEF unit in a biosafety level 2 laboratory.

Results: Inactivation of surrogate *E. coli* at 45, 50, and 55°C were 2.87, 3.11, and 3.55 log CFU/ml, respectively, in plain juice (pH 3.4), and 2.64, 3.24, and 5.05 with the addition of benzoic and sorbic acids (pH 3.5). Inactivation of *E. coli* O157:H7 under the same conditions were 3.03, 3.87, and 5.20 log CFU/ml, respectively, and 2.70, 3.63, and 5.70 with antimicrobials. *E. coli* O157:H7 in juice with antimicrobials and 2.7% citric acid (pH 2.6) treated with PEF was reduced by 3.88, 6.11 and > 6.11 log CFU/ml at 45, 50 and 55°C, while the surrogate E. coli decreased by 4.31, 4.93, and > 6.21 log under the same conditions.

Significance: Slightly greater inactivation of *E. coli* O157:H7 than of the surrogate bacterium indicates that *E. coli* ATCC 35218 provides a margin of safety when used as a surrogate for O157:H7 in strawberry juice treated with PEF. Further studies will be performed on scaled-up PEF equipment in a pilot plant.

P3-94 Inactivation of Mango Nectar Native Flora Combining Low Frequency Ultrasound and Short Wave Ultraviolet Light

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Introduction: The application of emerging technologies for food preservation has increased as an alternative to traditional thermal processing to supply safe food of high quality.

Purpose: The main objective of our research was to evaluate and model the survival of native flora in mango nectar (30% mango pulp, 12°Brix, pH 4.9, 0.29% titratable acidity) processed by short wave ultraviolet radiation (UVC), high intensity ultrasound (US), and their simultaneous application (UVC-US).

Methods: US treatments (20 kHz, 95 µm-wave amplitude) were performed by introducing a 13 mm probe in a jacketed 500mL-vessel at 15°C containing the inoculated system. The UVC device consisted of a 70 cm long glass tube fixed vertically with a 70 cm long UVC-lamp (30 W, 254nm, low pressure) inside the tube. Mango nectar was pumped through the UVC chamber using a peristaltic pump adjusted to 6 mL/s flow rate. Treated nectar was re-circulated from a 500 mL-vessel at 15°C. Microbial populations of inoculated samples were monitored by plate counting and spiral plate techniques during the treatments. In order to quantify the microbial response during the studied treatments, the Weibull type distribution of resistances model was applied. Model parameters were obtained using nonlinear regression.

Results: Initial mold and yeast count in the product was below the detection limit (< 10 UFC/ml) so their response during treatments was not modeled. UVC treatment was effective in suppressing the standard plate count in mango nectar reaching 3 log reductions in about 30 min. US was less effective but the simultaneous treatment reduced more than 4 log cycles after 30 min. Experimental curves were highly correlated to predicted data, obtaining significant determination coefficients. US survival curve showed a strong right skew distribution related to upward concavity (n < 1). UVC and UVC-US survival curves shown downward concavity (n > 1) and right skew distributions. A Weibull type distribution model was useful to explain observed differences among treatments.

Significance: The use of UVC combined with US was effective for enhancing inactivation of mango nectar native flora.

P3-95 Use of a Terbium-dipicolinic Acid Fluorescence Assay to Analyze Thermal Death Time Data of *Bacillus* and *Clostridium Endospores*

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Introduction: Clostridium spp. and *Bacillus* spp. cause foodborne illness and spoilage and form heat resistant endospores. The variation of spore resistance necessitates screening of large numbers of strains for validation of novel processes in food preservation. Dipicolinic acid (DPA) is a core constituent of endospores and is released during germination or breach of endospore ultrastructure. DPA release from *Bacillus* endospores generally correlates to spore inactivation by thermal treatments.

Purpose: This study aimed to assess thermal inactivation of endospores of *Clostridium beijerinckii* and *Bacillus amyloliquefaciens* using a terbium-dipicolinic acid fluorescence assay. This assay was also used to determine the impact of nisin on heat resistance of spores of *C. beijerinckii*.

Methods: Thermal inactivation kinetics were established for endospores of *B. amyloliquefaciens* FAD11/2 and *C. beijerinckii* ATCC 8260 incubated in 0.9% NaCl solution at 90°C, 100°C, and 120°C for 0 to 60 min. The minimum inhibitory concentration of nisin against *C. beijerinckii* spores was determined by a critical dilution assay and spores of the organism were treated at 100°C for 0 to 60 min in presence of 16 mg/L nisin. Thermal inactivation curves were analyzed by determination of the DPA release with the terbium-dipicolinic acid assay and the relationship between counts of viable spores on RCM agar and DPA release was determined for *B. amyloliquefaciens*.

Results: The rate of DPA release from spores correlated well to the inactivation of spores for *B. amyloliquefaciens* at 90°C, 100°C and 120°C, and increased with temperature of processing for *C. beijerinckii*. The strain was inhibited by 0.6 mg/L and 16 mg/L nisin enhanced DPA-release from *C. beijerinckii* by lethal heat treatments.

Significance: The fluorescence assay to determine DPA release may be suitable for a rapid screening of resistance to heat and bacteriocins of *Clostridium* spp. spores.

Poster

P3-96 Survival of Lactic Acid Bacteria in Various Water Sources and Sandy Loam Soil
DSC

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Introduction: Lactic acid bacteria are known to have antagonist properties that are capable of reducing *E. coli* O157:H7 in multiple environments. Soil and water has been identified as vectors for transmission of *E. coli* O157:H7 onto fresh produce and onto live cattle.

Purpose: The objective of this study was to evaluate the survival of lactic acid bacteria (LAB) in three types of water sources (tap water, well water, and autoclaved water) and in sandy loam soil to determine if water and soil applications could serve as interventions.

Methods: Lubbock municipal tap water (very hard, 289 ppm), a local farm well water (moderately hard, 110 ppm) and autoclaved softened water (soft, 40 ppm) and local sandy loam soil were inoculated separately with a lactic acid bacteria 5-strain inoculum at a 10^8 and 10^{10} CFU/(ml/g) load, respectively. Water samples and soil were placed within a plant growth chamber set at fall in central California for 48 h (0, 6, 12, 18, 24, 48 h) or 28 days (0, 1, 7, 10, 14, 21, 28 days), respectively. On each sample day, samples were diluted, plated onto MRS and incubated at 37°F for 48 h. The data were analyzed using a descriptive analysis in SAS program.

Results: In the water study, the tap water maintained a significantly lower LAB count than the other water sources, regardless of the sampling time ($P < 0.03$). Although there was a sampling time effect reported ($P = 0.0325$), all water sources had less than 0.8 log CFU/ml reductions of LAB found over the 48-h study. Within the soil study, the lactic acid bacteria were significantly reduced by 3.0 log cycles by the end of the study. LAB reduction was found within the first week ($P < 0.02$) and another log CFU/g was found between day 21 and 28 ($P < 0.001$).

Significance: Water applications of LAB will likely be performed within 48 h. According to this study, addition of a 1 log higher dose of the LAB to the water might be necessary to ensure proper application rates, depending on the water type. In sandy loam soil, a 1 log increase in the initial population should be added as well, and this should give a protective effect for up to 21 days before another application of the LAB would be needed. Use of LAB in the soil and water has potential application in produce and animal environments to reduce foodborne pathogens.

P3-97 Withdrawn

P3-98 Application of a Novel Single Bacterial Cell Manipulation Technique *Listeria monocytogenes*
DSC

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Introduction: Highly diluted suspensions of bacterial cells are distributed according to the Poisson distribution. Quantitative microbiological methods (Most Probable Number, MPN) are based on this prerequisite. Nevertheless, predictions on the growth performance of single bacterial cells are not possible due to these statistical effects. A new method that avoids these influences was developed which enables the physical manipulation of single bacterial cells. Based on this method the investigation of autonomous growth of low bacterial cell inocula (< 10) was performed.

Purpose: The purpose of this study was to evaluate the growth performance of single bacterial cells without the influence of statistics within dilution series. Furthermore, the necessity of chemical and physical cell-to-cell interactions for bacterial growth was investigated.

Methods: Listeria monocytogenes EGDe bacteria from the lag-phase, the mid-exponential phase, and the stationary phase were used to produce single cell inocula with the newly developed single bacterial cell manipulation technique (SBCM). Growth in tryptone soy broth with 0.6% yeast was evaluated after 24 h at 37°C by measurement of optical density and by selective and non-selective plating.

Results: For 110 manipulated single cells, growth was detectable in 79 samples (71.5%), with a final optical density of 1.21×10^9 CFU/ml (± 9.07%). In 31 samples (28.5%) growth was not detectable. The live/dead ratio of the initial culture was 20.9% (± 20.6%) as obtained by live/dead staining. These results show a good correlation of live/dead ratios before and after the SBCM, indicating the ability for autonomous growth.

Significance: These data suggest that the investigated single bacterial cells are able to multiply independently under optimal conditions.

P3-99 Evaluation of a New Automated Method for Enumerating Yeast and Mold in Food Products

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Introduction: Yeast and Mold (YM) enumeration in food is useful in evaluating its quality and the degree of deterioration and is often an essential component in microbiological quality assurance programs.

Purpose: In this study, we compared the TEMPO automated method for the enumeration of YM to the FDA-BAM recommended procedure: Dichloran rose bengal chloramphenicol (DRBC) or Dichloran 18% glycerol agar (DG18) media depending on the food product a_w, and to Potato Dextrose Agar (PDA).

Methods: TEMPO™ enumeration is based on the well known Most Probable Number (MPN) procedure. The method uses a selective dehydrated culture medium and an enumeration card for the automatic determination of the MPN. A total of 400 naturally contaminated products were tested. These products represented a wide range of food categories, including dairy products, raw and cooked meats, poultry, fish and seafood, vegetables, Ready-to-Eat foods (RTE) and environmental

Poster

samples. For some discrepant results, some confirmation tests were performed to distinguish between bacteria or yeast. A combination of regression analyses, difference log distributions and *t*-tests at the 5% level were used to analyse the data and compare performances.

Results: This automated method showed similar performances to the BAM reference methods with good agreement on the whole data. Regression analysis and *t*-tests show a slight negative bias compared to DRBC/DG18 due to a better selectivity of TEMPO YM, confirmed by the complementary tests performed from DRBC and DG18. The comparison with PDA does not show bias significantly different from 0.

Significance: The results suggest that the PDA, DRBC/DG18 and TEMPO YM methods are equivalent for enumerating yeast and mold. The automated method offers food laboratories a rapid alternative for YM enumeration with a time to result of only 3 days, compared to 5 days for FDA-BAM.

P3-100 Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) of the aroA Gene from *Arcobacter butzleri* Korean Isolates

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Introduction: The aroA gene encodes 5-enolpyruvylshikimate-3-phosphate synthase which contributes to aromatic amino acid and folic acid pathway in bacteria. This gene is often used for the typing, detection, and taxonomic analysis of pathogens. Although complete genome sequence of *Arcobacter butzleri* (RM4018) was reported in 2007, the diversity of aroA gene from *A. butzleri* field isolates was not known.

Purpose: We aimed to investigate the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) patterns of the aroA gene amplified from *A. butzleri* Korean isolates.

Methods: Thirty-nine *A. butzleri* were isolated from Korean chickens and confirmed by multiplex polymerase chain reaction. We designed the specific primer set for the aroA gene of *A. butzleri*, using Primer3. PCR products for aroA were digested with Sau3A or HindIII restriction enzyme. Digested DNA products were analyzed with DigidocIT program.

Results: We obtained 1,025bp length DNA fragments from all isolates by aroA-specific PCR. Two distinct patterns were identified in PCR-RFLP with Sau3A. In the results with PCR-RFLP with HindIII, four distinct patterns were observed.

Significance: PCR-RFLP of the aroA gene with Sau3A and HindIII restriction enzyme is a useful tool for identifying and typing *A. butzleri*.

P3-101 Subtyping and Characterization of *Cronobacter sakazakii* Isolated from Powdered Food

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Introduction: Cronobacter sakazakii has been associated with necrotizing enterocolitis, bacteremia, and infant meningitis through the ingestion of contaminated powdered infant formula. *C. sakazakii* was defined by Farmer et al. and Iversen et al. who described 16 biogroups according to the biochemical characterization.

Purpose: This study was performed with biogrouping and genotyping by biochemical characterization, 16s rDNA analysis and physiological property of *C. sakazakii* isolated from powdered foods.

Methods: 46 strains of *C. sakazakii* were isolated and identified by conventional culture method, biochemical test, PCR and sequencing.

Results: As the results of genotype and biogroup of isolated *C. sakazakii*, Cluster I group (biogroup 1-5, 7-9, 11, 13 and 14) was composed of the major isolates and five isolates were belonged to cluster IV group (biogroup 6,10 and 12). Particularly, eleven isolates were confirmed, with new biogroup 17 showed different biochemical profiles such as motility (-) and nitrite (-). However, through the comparison of systematic homology, new biogroup 17 was found to belong to Cluster I group. Also, as the results of dry resistance by Cluster group, about 31 % of cluster I group was very tolerant to dry, but all of cluster IV group was sensitive to dryness. Biofilm formations of the dry tolerant *C. sakazakii* were early formed than dry sensitive isolates.

Significance: Thus, a new biogroup 17 was confirmed with Cluster I group and dry-resistant isolates among the Cluster I group formed biofilms more rapidly than than dry-sensitive isolates.

P3-102 Rapid Two Day Isolation and Identification of *Salmonella* Using Single Selective Enrichment and Brilliance™ *Salmonella* Agar

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Introduction: Salmonella is a Gram-negative, rod-shaped, motile bacterium with a widespread occurrence in animals, especially in poultry and swine. Additional sources of this organism include raw meat, raw poultry, and raw seafood, to name only a few. Detection is critical as *Salmonella* is the most frequently reported cause of foodborne illness (40,000 to 50,000 cases reported annually) and the infectious dose can be as low as 1 to 10 CFU/g.

Purpose: This study evaluates the Oxoid Salmonella ONE Broth for enrichment of food samples and plating on Brilliance™ Salmonella Chromogenic Medium (formerly referred to as Mark II) as a detection method for Salmonella in at-risk food matrices.

Methods: Ground beef, ground chicken, lettuce, shrimp, and shell eggs were inoculated with *Salmonella* serovars at a level of ~1 CFU/25 g. Samples were then enriched in Oxoid Salmonella ONE Broth, plated on Brilliance™ Salmonella Chromogenic Medium, and compared to either the USDA or FDA established protocols. Additionally, this method was evaluated on the performance of specificity using multiple *Salmonella* serovars n = 100) or closely related bacterial species (n = 30).

Results: When selected foods were inoculated with *Salmonella* serovars at a level of ~1 CFU/25 g, there was no difference in sensitivity between this method and the standard reference methods. When specificity was evaluated 96/100 *Salmonella* serovars were identified using this method, and 29/30 of the non-*Salmonella* showed no growth or atypical growth.

Significance: Enriching samples using ONE Broth Salmonella and plating on Brilliance™ Salmonella medium reduced time to detection to 2 days, compared to 4 days for the FDA and USDA methods.

P3-103 Purification and Characterization of Xylanase from a New Strain of *Bacillus* sp.

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Introduction: Xylo-oligosaccharides are non-carcinogenic and considered to be prebiotics that can promote the growth of beneficial bifidobacteria in the colon. They are sugar oligomers produced during hydrolysis of xylan, a major component of plant hemicellulose. Xylanases are glycosidases that can catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan.

Purpose: The aim of this study was to purify and characterize xylanase produced by a new strain *Bacillus* sp. grown on rice straw.

Methods: The cells of *Bacillus* sp. were removed by passing through a 0.45 μm membrane after 4 days incubation at 25°C which had the highest xylanase activity (2.26 U/mL). Purification procedures included ammonium sulfate precipitation, ion exchange and gel filtration chromatographs.

Results: The xylanase was purified to electrophorectical homogeneity by precipitating at 40 to 60% saturation of ammonium sulphate, passing through CM-Sepharose FF and Sephacryl S-100 HR chromatographs. About 3.5% of xylanase was recovered and 678.1-fold purification was obtained. The purified xylanase was with a molecular mass of 19 kDa and specific activity of 1435.98 U/mg. It had an optimal pH and temperature at 6.0 and 50°C, respectively, and was stable at pH 5.0~9.0 and 10~40°C. This xylanase was highly inhibited by Cu^{2+}, Fe^{3+}, Hg^{2+}, PMSF, TPCK, NEM and Leupeptin, but strongly activated by β-mercaptoethanol and glutathione.

Significance: According to substrate specificity, the purified xylanase had high specificity to beechwood xylan, birchwood xylan and oat spelts xylan. From the above substrate specificity suggests the potential use of the purified xylanase in xylooligosaccharide production.

P3-104 Survival of *Escherichia coli* O157:H7 and *Salmonella* Newport in Animal Feces

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Introduction: Human enteric pathogens can be shed in animal feces, which in turn could serve as the primary source for contamination of vegetables in the field. *Escherichia coli* O157:H7 and *Salmonella enterica* are well known to survive in animal feces. The survival of these pathogens in animal feces shed in vegetable fields can be affected by several factors, including relative humidity (RH) and temperature.

Purpose: The objective of this study was to determine the growth and/or survival of *E. coli* O157:H7 and *S. enterica* serotype Newport in different animal feces exposed to various temperatures and RH levels.

Methods: Cow and dog feces were inoculated with *E. coli* O157:H7 or *S.* Newport overnight culture ($10M^7$ CFU/g). Samples were stored at either 26°C, 40% RH or 15°C, 80% RH. Surviving populations of *E. coli* O157:H7 and *S.* Newport were enumerated by taking samples at different times, stomaching, diluting, and plating on appropriate media.

Results: In dog feces, both *E. coli* O157:H7 and *S.* Newport survived longer at 15°C, 80% RH than at 26°C, 40% RH. Both pathogens survived up to 7 days at 15°C, while the survival was only 3 days at 26°C. With one batch of cow feces, the survival of *E. coli* O157:H7 was 10^3 CFU/g on day 370 at 26°C, and 10^2 CFU/g on day 373 at 15°C. The survival of *S.* Newport was 10^2 CFU/g on day 289 at 15°C, as well as on day 284 at 26°C. In another batch of cow feces, for both pathogens, no survivors were detected on day 180 at 15°C, but both pathogens survived longer than 230 days (10^2 CFU/g) at 26°C.

Significance: This study will provide the produce industry with a good scientific basis to understand pathogen survival in animal feces under different conditions, which can in turn contribute to mitigating microbiological risks from contaminated vegetables.

P3-105 Recovery and Infectivity of Norovirus in Bacterial Biofilms on Stainless Steel

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Introduction: Recurring outbreaks of norovirus infection have occurred among individuals in close association despite reported fervent cleaning of common contact surfaces. The persistence of hepatitis A virus and bacteriophage in bacterial aggregations in food and drinking water systems, respectively, has been observed. The persistence of norovirus in biofilms has not been reported.

Purpose: The incorporation and infectivity of mouse norovirus (MNV-1), as a surrogate for human norovirus, was assessed on material commonly found in food handling environments.

Methods: Duplicate stainless steel (type 304, 16-gauge) coupons (1 in by 3 in) were suspended in 25 ml deionized water with MNV-1 (10^4 PFU/ml) alone or in a cocktail of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli* (3×10^7 CFU/ml combined) added either simultaneously or 6 h prior to the addition of virus. Inoculated coupons were incubated unagitated at ambient temperature for 48 h. Coupons were rinsed in deionized water stream (20 ml), then shaken vigorously (30 s) in 25 ml of Butterfield's Phosphate Buffer (BPB). Bacteria recovered in BPB were enumerated on TSA. BPB was centrifuged to pellet bacteria, and infectivity of MNV-1 in the supernatant was determined by plaque assay in RAW cells. The efficacy of Virkon® (0.1 g/mL, 10-minute submersion) disinfection on coupons was evaluated.

Results: Infectious MNV-1 was not recovered from the stainless steel coupons (10 PFU/ml detection limit) whether integrated simultaneously or after 6 h of biofilm formation. The bacterial population recovered from the coupons was approximately 10^3 CFU/ml except after disinfectant treatment, from which < 10 CFU/ml were recovered.

Significance: Detection of infective MNV-1 from 48-h biofilms on stainless steel was not possible with the low inoculum and recovery methods employed. Current investigations include evaluation of higher initial virus populations and different recovery and detection methods on determination of virus integration and survival in bacterial biofilms.

P3-106 Antimicrobial Activities of Cinnamaldehyde and Carvacrol against *Salmonella* Newport on Contaminated Oyster and Celery

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Introduction: Consumers generally prefer natural over synthetic food additives. Safe plant extracts such as essential oils, their active components, and spices have been shown to exhibit antimicrobial effects against foodborne pathogens.

Purpose: The objective of this study was to define the antimicrobial activities of cinnamaldehyde (the main ingredient of cinnamon oil) and carvacrol (the main ingredient of oregano oil), against *Salmonella* Newport on oysters and celery.

Methods: Oyster or celery samples were dipped in *S.* Newport culture (10^7 CFU/ml) for 2 min, and dried for 1 h. Celery samples were dipped in 1% cinnamaldehyde or carvacrol in phosphate buffered saline (PBS) for 10 min and oyster samples for 10 min or 1 h. All samples were stored at 4°C for 3 days. Enumerations of surviving bacteria were done at days 0 and 3.

Results: When samples were dipped in 1% carvacrol for 10 min, no survivors were detected on celery at day 0. There were about 1 and 1.5 log reductions on oysters at days 0 and 3, respectively. Oyster samples dipped in carvacrol for 1 hr showed about 1.5 and 5 log reductions at days 0 and day 3, respectively. Celery samples exposed to cinnamaldehyde showed 1 and 2.5 log reductions at days 0 and 3, respectively. The bacterial count on oysters exposed for 10 min to cinnamaldehyde was reduced by about 1 and 2 logs at days 0 and 3, respectively. Oysters exposed for 1 hr to cinnamaldehyde had 1 and 5 log reductions at days 0 and 3, respectively. Dipping test samples in PBS (control) had no effect on *Salmonella*.

Significance: This study demonstrates the potential of carvacrol and cinnamaldehyde to inactivate *S.* Newport on contaminated celery and oysters. The potential of these compounds for inactivation of *S.* Newport and other pathogens on produce and seafood merits study by the food industry.

P3-107 Risk Factors Associated with the Presence of *Listeria* in Rural Households with or without Ruminant Animals

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Introduction: Ruminants may be a reservoir for *Listeria* and a potential source of household contamination. However, there are no available data on how *Listeria* could be introduced in the household environment. Understanding consumer behavior may help in development of education materials on preventive measures to further reduce the risk of listeriosis.

Purpose: This study evaluated consumer behaviors in households with or without ruminants, associated with cleaning, food handling and storage, which may be related to increased prevalence of *Listeria* in the household environment.

Methods: Rural Colorado households (27 with and 27 without ruminants) were recruited, samples (food, environmental, and human and animal feces) were collected four times (at 2 to 3 week intervals), and tested for *Listeria*. Participants answered surveys regarding cleaning habits, food handling (storage, preparation and preferences), and animal handling. The study was completed over a three-year period, with samples collected during years one and three. Seven indices were developed from survey information, and were statistically analyzed for relationships to the outcome with a sample positive for *Listeria* as the dependent variable.

Results: Listeria was isolated from all types of samples with higher prevalence in households with ruminants. Compared to year-1, prevalence in food, refrigerators and washing machines was higher in year-3, and prevalence on kitchen sinks, shoes, working gloves and ruminant feces was lower. The perishable food handling and cooking index affected ($P < 0.05$) the probability of households testing positive for *Listeria*, regardless of presence of ruminants. For households with ruminants, personal cleanliness habits were related to presence of *Listeria* on shoes, clothes washing machine, and working gloves. Shoes testing positive in ruminant households were more frequently associated with multiple positive environmental samples, when compared to households without ruminants.

Significance: Consumer education on handling and storing perishable foods and on animal handling to prevent contamination of the household through shoes or clothes may reduce prevalence of *Listeria* in home environments.

TECHNICAL ABSTRACTS

T1-01 Development of a Risk-based Approach for Regulation of Raw Milk Products in New Zealand

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Introduction: Currently, only limited varieties of cheeses manufactured from raw milk are imported and sold in New Zealand following case-by-case risk assessments. All other imported dairy products, and all dairy products manufactured and sold in New Zealand, must be pasteurized or thermized. There has been increasing interest in producing raw milk products within New Zealand and in importing a greater variety of products, particularly cheeses.

Purpose: The New Zealand Food Safety Authority (NZFSA) sought to develop a legislative framework to allow domestic manufacture and sale of raw milk products. In so doing, the major issues addressed were: 1. The nature of the legislative framework required to support a risk based approach to regulating raw milk products. 2. Potential additional requirements that were needed to achieve an appropriate level of safety for raw milk products.

Methods: Reviews of international risk assessment and management for raw milk products were undertaken, including relevant international guidelines such as those developed by the Codex Alimentarius Commission. Factors used to determine risk were knowledge of intrinsic product characteristics, the processing steps used for manufacture and available risk assessment studies. Additional science work was commissioned to establish the impact that additional requirements would likely have on the safety of raw milk products, and modeling tools were developed to assist in risk determination and categorization of products.

Results: The proposed regulatory framework defined three broad categories of raw milk product based on public health risk. Category one products were deemed to present a level of safety similar to that of pasteurized or thermized milk products, and no further regulatory requirements were necessary. Category two products, although considered to present a slightly higher risk to the general population, presented an acceptable level of safety but were subject to additional on-farm safety requirements and, in some cases, demonstration of an effective and validated manufacturing process. Category three products were considered to present an unacceptable risk to public health and will not be permitted to be manufactured without detailed studies demonstrating that they meet an appropriate level of safety.

Significance: The regulatory framework and associated risk management tools will allow more efficient and effective regulation of a range of raw milk products in New Zealand.

T1-02 Effect of Cooling Rate and Natural Antimicrobials on Growth of *Listeria monocytogenes* in Cottage Cheese

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Introduction: The US Pasteurized Milk Ordinance (PMO) requires that dairy products be filled and stored at 7°C (45°F). However, commercial cold-filled cottage cheese curd and dressing are frequently mixed and filled at temperatures as high as 12.8°C (55°F); cooling to 7°C may require as long as 72 h.

Purpose: To evaluate the effect of natural microbial inhibitors on the growth of *Listeria monocytogenes* (*Lm*) in cold-filled cottage cheese during rapid and extended cooling.

Methods: Full-fat (4% fat) and reduced-fat (1% fat) cottage cheese (pH 5.25) were supplemented with microbial inhibitors. Eight antimicrobial treatments were evaluated, including Control (no antimicrobials), 0.03 or 0.04% Bioactive Protein (BP), 0.15% Fermentate D, 0.1% Fermentate E with or without Live Culture-E, and 0.3 or 0.5% Fermentate A. Inhibitors could be labeled as cultured milk solids, calcium lactate, or are exempt from labeling as incidental additives. Products were inoculated with 3.5 log CFU/g *Lm* and 100 g portions packaged into polypropylene vials. One set of packages was immediately chilled to 7°C, whereas a parallel set of packages was cooled from 12.8°C to 7°C within 72 h; both sets were stored at 7°C for the remainder of the two-week study. Triplicate samples of each treatment were enumerated by plating on Modified Oxford agar at 0, 1, 2, 3, 4, 7, 10, and 14 days storage.

Results: Populations of *Lm* increased 0.23 + 0.15, 1.55 + 0.33, 3.44 + 0.39, and 4.83 + 0.19 log at 4, 7, 10, and 14 days, respectively, in Control cottage cheese stored at constant 7°C. Controls cooled slowly supported > 1 and > 3 log increase in 4 and 7 days, respectively. In contrast, the addition of inhibitors delayed or prevented growth of *Lm* in cottage cheese cooled slowly compared with the Controls. Approximately a 1-log increase of *Lm* was observed in slow-cooled cottage cheese supplemented with 0.15% Fermentate D or 0.1% Fermentate E treatments at 7 days, whereas no growth was detected at day 7 for 0.03% BP, day 10 for 0.04% BP or 0.3% Fermentate A, or day 14 for 0.5% Fermentate A or 0.1% Fermentate E + LiveCultureE.

Significance: This study demonstrated that in cottage cheese with initial pH 5.25, certain natural microbial inhibitors will delay or prevent the growth of *L. monocytogenes* during slow cooling from 12.8 to 7°C in 72 h, followed by storage at 7°C for up to two weeks. In addition to these measures, strict adherence to good manufacturing practices and good environmental monitoring program is essential to ensure product safety.

T1-03 DSC Evaluating the Potential for Translocation of *Listeria monocytogenes* from Floor Drains to Food Contact Surfaces in the Surrounding Environment Using *Listeria innocua* as Surrogate

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Introduction: Floor drains in processing environments harbor *Listeria* spp. due to continuous presence of humidity and organic substrates. The cleaning and washing activities undertaken in the processing facilities may translocate the bacterial cells from the drain into the surrounding environment thus contaminating food products being produced.

Purpose: This study evaluates the potential for translocation of *Listeria monocytogenes* from drains to food contact surfaces in the surrounding environment using *Listeria innocua* as a surrogate.

Methods: A 7 × 7 × 8 foot flexi-glass chamber was built in which a cast iron drain mounted on an aluminum cabinet was placed. Stainless steel coupons (6.4 × 1.9 × 0.1 cm, 12 per height) were hung at 3 different heights 1, 3 and 5 feet inside the chamber. The drain was inoculated with meat slurry (10 g ground beef/liter water, and 6 to 7 log CFU/ml of *L. innocua* four strain cocktail) and a commercial cleaner and a sanitizer was used. Cell translocation from the drain onto the coupons due to aerosols generated during cleaning and washing of drain was studied. Coupons were incubated in *Listeria* enrichment broth for 48 h. Turbid broths were streaked onto modified oxford medium (MOX) agar. Typical colonies that appeared on MOX plates were confirmed using VIP Listeria rapid test. For statistical analysis, Single Factor Model with binomial distribution was used and data were analyzed using GENMOD procedure in SAS.

Results: Significantly higher translocation ($P < 0.05$) was seen at 1 foot (up to 25%), followed by 3 feet (up to 11%) and 5 feet (up to 2.7%) indicating that the closer the surface from the drain, the greater the number of bacterial cells that transfer from the drain to the surrounding surfaces.

Significance: Results show that *L. monocytogenes* may translocate from drain to food contact surfaces via aerosols generated due to cleaning and washing, thus contaminating food products.

T1-04 Sanitizing Efficacy on Sessile and Planktonic *Listeria monocytogenes* Cells

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Introduction: Bacterial attachment and the subsequent biofilm formation on various environmental surfaces in food manufacturing is an important contributor to *Listeria monocytogenes* contamination of final food products. Even with intensive sanitation regimes, biofilms containing *L. monocytogenes* can remain on environmental surfaces, resulting in increased resistance to chemical sanitizers.

Purpose: The objective of this study was to assess the bactericidal concentration of 24 sanitizing agents against 20 *L. monocytogenes* strains.

Methods: All strains were inoculated in tryptic soy broth containing 0.6% of yeast extract. Planktonic cells were incubated for 24 h at 37°C and biofilms for 48-h at 30°C. Biofilms were rinsed with water to remove non-attached cells. Sanitizers were diluted in hard water (200 ppm), using the manufacturer's instructions. Experiments were conducted in microtitre polystyrene plates.

Results: The results showed that all sanitizers were effective against planktonic *L. monocytogenes* cells at the manufacturer's recommended concentrations. With respect to *L. monocytogenes* biofilms, only the peroxyacetic acid, chlorine dioxide and acidified chlorine-based sanitizers were able to achieve a 5 log reduction of viable bacterial cells at the recommended concentrations after 5 minutes of contact time. Independent of the PFGE profile, serotype, ability to form biofilm or source (human, environment or food) of the isolates, they all showed similar responses to the sanitizers.

Significance: This study suggests that biofilms are more sensitive to certain chemicals with high oxidizing power. No relationship was observed between the chemical susceptibility of the isolates and the other intrinsic characteristics investigated.

T1-05 DSC Efficiency and Optimization of UV Exposure to Reduce *Listeria monocytogenes* Contamination on Conveyor Belts Made from Four Different Materials

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Introduction: Listeria monocytogenes (LM) has been repeatedly isolated from foods and food processing facilities. LM can harbor on conveyor belts (CB) and disseminate on food. CB are often difficult to clean and involve rigorous sanitation programs to get rid of bacteria. Ultraviolet (UV) light has exhibited microbicidal properties on food and food contact surfaces. Disinfection efficiency of UV depends on bacterial species, their growth stage and surface of attachment. There is little published information on the use of UV to disinfect CB.

Purpose: This study was conducted to determine efficiency and optimize parameters for the use of UV to kill LM on CB made of four different belting materials.

Methods: A cocktail of five LM isolates (serotypes 3, 4b, 4c and strains 108M, 109) was made to give a suspension of approximately 10^6 CFU/mL. The suspension was inoculated on four types of CB; (1) Ronanyl DM 8/2 A2 + 04, (2) Volta FRMW-3.0, (3) Volta FRMB-3.0 and (4) Ropanyl DM. Each inoculated CB was subjected separately to UVC (254 nm) for 1 and 3 s at 5.95 mW/cm^2 and 5.53 mW/cm^2. Effect of bacterial age on disinfection efficiency was determined by exposing 12 h and 24 h old culture suspensions for 1, 3 and 5 s at 3.83 mW/cm^2 and 3.52 mW/cm^2 intensities on one type of belt (CB1). Analysis was conducted in triplicates and results analyzed using SAS.

Results: LM count was significantly reduced on all the belts irrespective of energy intensities and time of exposure. No bacterial colonies were recovered on exposure of CB1 and 2 for 3 s at an energy intensity of 5.93 mW/cm^2 CB 1 and 2 exposed to 5.95 mW/cm^2 showed a higher survival (0.85 to 2.29 log CFU/cm^2) as compared to 5.93 mW/cm^2. CB 4 showed a survival of 1.62 to 2.39 log CFU/cm^2. Maximum reduction in LM count (2.1 log CFU/cm^2) was observed in 12 h culture at 3 and 5 s at 3.83 mW/cm^2.

Significance: UV can be effectively used to reduce the risk of LM contamination via CB.

T1-06 Enterobacteriaceae and Related Organisms Recovered from Biofilms in a Commercial Shell Egg Processing Facility

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Introduction: Effective sanitation programs in shell egg sanitating facilities are essential to the production of wholesome eggs and decrease the risk of egg-borne disease.

Purpose: Determine the identity of bacteria that contribute to biofilm formation in shell egg processing facilities and on egg and non-egg contact equipment surfaces.

Methods: During six visits, biofilms from egg contact and non-contact surfaces in a commercial shell egg processing facility were sampled. Thirty-five different sample sites were selected: belts (farm, accumulator)pre-wash and wash tanks (lids, screens, tank interiors, nozzle guards, spindles), post-wash spindles, blower filters, scales, packer lanes (belts, brushes, sorters), plastic flat washers, and floor drains. Whirl-pak sponges moistened with 10 mL D/E neutralizing broth were used for aseptic to biofilm collection from each sample site. At the laboratory, aliquots were pour plated using violet red bile glucose agar (VRBG) with overlay and incubated at 37 C for 18 to 24 h. Enumerated results were described in a previous report. Isolates (1 to 3/plate) were selected randomly from plates exhibiting typical presumptive colonies. To increase the chance of isolate purity, each selected colony was restreaked three times on plate count agar incubated at 37°C for 18 to 24 h. An automated system using 95 biochemical tests was used to determine identifications to genus or species for 269 isolates.

Results: Enterobacteriaceae genera recovered accounted for 91.4% (246/269) of identifications and included *Buttiauxella*, *Cedecea*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Morganella*, *Pantoea*, *Proteus*, *Providencia*, *Rahnella*, *Raoultella*, *Salmonella*, *Serratia*, *Shigella*, and *Xenorhabdus*. Other genera recovered accounted for 8.6% (23/269) of the identifications and included: *Aeromonas*, *Aquaspirillium*, *Pseudomonas*, *Vibrio*, and *Ralstonia*. As has been reported previously, *Enterobacter* and *Escherichia* accounted for a majority of the identifications, 15.2 and 25.6%, respectively. *Citrobacter*, *Klebsiella*, and *Raoultella* accounted for 11.5, 12.6, and 11.2% of identifications. *Aeromonas* and *Vibrio* were associated with wash tank samples. *Salmonella* isolates were recovered from packer lane samples used to pack defective eggs (cracked shells, dirties, etc.) for shipment to egg breaking facilities.

Significance: Understanding which genus or species contribute to biofilm contamination in commercial facilities can be used to develop more effective sanitation programs.

T1-07 Identification of a Unique Food Safety Risk Associated with Retail Markets Serving Asian Populations in America DSC

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Introduction: Tofu is consumed by many and is especially popular with Asian populations. Previous research has examined its safety and found that if not handled properly, it may present a food safety risk. In this research, we examined the safety and prevalence of a practice in small Asian urban markets to sell fresh tofu from a "bucket" or open bin from which consumers who wish to purchase the product, remove the tofu cake and place it in a plastic bag.

Purpose: This study examined the prevalence of this practice within the city of Philadelphia and the safety of the product sold using this practice as compared to traditionally pre-packaged tofu sold in the same markets.

Methods: US Census Bureau data was used to identify tracts with high Asian populations in Philadelphia. Two databases were used to identify food store outlets in those tracts. Approximately 150 stores were identified and visited, and fifteen were found to practice the sale of tofu from "buckets" or open bins. These 15 stores were visited on multiple occasions and both "bucket" as well as prepackaged tofu samples obtained. Both tofu cakes and juice were tested for temperature, pH, Aerobic Plate Count, coliforms, fecal coliforms, *E. coli, S. aureus* and *L. monocytogenes*.

Results: Results for 37 tofu samples and juice sold from the bucket were compared to 14 prepackaged samples and their juice. For tofu samples from buckets and packaged tofu, respectively: 73% and 28% had an aerobic plate count above 10^6 CFU/g; fecal coliforms were found in 19% and 7%; average temperature of storage was 49 and 45°F; presence of *S. aureus* was found in 3% of tofu from buckets; *E. coli* was found in 5% of juice from buckets.

Significance: This study identified a common risky food handling practice for the sale of tofu in small Asian retail markets.

T1-08 Sanitation Schedules – A New Management Approach

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Introduction: The continuing growth of the Ready-to-Eat (RTE) sector and the increasing desire to more effectively manage the sanitation processes to control pathogens, has led to a food industry review in the UK as to determine how best to document and manage sanitation, both on a routine basis and in response to a potential, environmental pathogen contamination incident.

Purpose: A working party of food manufacturers, cleaning chemical suppliers, food retailers and Campden BRI met over the course of 18 months to optimise the management of the sanitation process, develop a number of sanitation schedules and propose guidance to food manufacturers on how the processes and schedules could be implemented.

Methods: Sanitation schedules were developed that document the necessary work instructions that allow the successful completion of the sanitation task. The number of sanitation schedules required will depend on the size of the manufacturing plant, the risk or category of the food product and the complexity of the equipment to be cleaned. Whilst only 2 or 3 may be required for traditional food processors, up to 6 schedules can be adopted for RTE manufacturers, described as cleaning plan, wholeroom, in-production, end of production, periodic and site decontamination.

Results: The cleaning plan lists all the sanitation tasks and when they should be undertaken. The wholeroom schedule details all necessary sanitation requirements (manpower, equipment, chemicals, operative safety, room preparation, prevention of recontamination, etc.) and the sequence of sanitation events designed to maximise the removal of microorganisms from the processing area. In-production schedules define housekeeping and safe product line changeover activities, whilst End of production schedules detail the major control of food soiling and microorganisms on a day-to-day basis. Periodic schedules define additional sanitation requirements, performed less frequently, to reduce microbial harbourage and biofilm formation, whilst site decontamination schedules are implemented only when environmental pathogen contamination is identified.

Significance: A sanitation management system has been produced to better manage food safety and that allows food manufacturers to detail all aspects of the sanitation process, which operatives can be trained against and which can be used as an auditing tool.

T1-09 Wholeroom Disinfection: A New Concept in Food Industry Sanitation

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Introduction: The traditional approach to controlling environmental contamination in the food industry has been to target specific sites within the processing environment (e.g., production equipment and utensils) with cleaning and disinfection regimes, which has been sufficient to maintain day-to-day control. In some instances, however, microbial strains have become persistent in food factories, surviving for several years.

Purpose: This work sought to assess the use of ozone (O_3) and hydrogen peroxide (H_2O_2) to control microorganisms on all environmental surfaces, an approach termed 'wholeroom' disinfection

Methods: Microbial suspensions dried onto stainless steel coupons were placed at a range of heights and orientations in a specifically designed aerobiology containment rooms. H_2O_2 was used only at 30% whilst trials with (O_3) were undertaken at concentrations below and above 10 ppm to simulate continuous daily operational and single use decontamination strategies as used in the UK. The use of O3 for up to 4 weeks in continuous factory-based trials was also assessed.

Results: In laboratory trials vapourized H_2O_2 achieved a 0.93 to 1.77 log reduction against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and a > 5.83 log reduction of *Bacillus globigii* and *Geobacillus stearothermophilus* spores. The enhanced disinfection of spores may be vapour concentration related as when *S. aureus* and *P. aeruginosa* were exposed to H_2O_2 in disinfectant suspension tests, log reductions were reduced from > 5 at 15% to < 3.9 at 5% H_2O_2, possibly due to catalase activity. A 0.5 and 1.15 log reduction of *S. aureus* and *P. aeruginosa* respectively was achieved with 8 ppm gaseous O_3 after 30 min exposure whilst 10 ppm for 35 to 45 min produced typically 2 to 3 log reductions for *Escherichia coli*, *P. aeruginosa*, *Listeria monocytogenes* and MRSA. Coupon orientation had no significant effect. Field trials demonstrated a 0.69 log reduction in environmental surface total viable count when O_3 gas replaced chemical disinfection, a performance equivalent to or better than previously obtained with quaternary ammonium compounds.

Significance: Wholeroom disinfection programmes may have a role to play in the food industry in daily post production sanitation programmes or for one-off applications to control environmental contamination.

T1-10 The Microbiological Safety of Irradiated Food

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Introduction: Food irradiation remains an emotive issue, despite over 100 years of research. Consumer resistance to food irradiation is founded in five main areas, of which microbiological safety was the focus of this review. Irradiation remains the most feasible technology for decontamination of many foods such as ready to eat produce and raw meat as it does not lead to any temperature rise within the food being treated.

Purpose: The aim of this review was to investigate the current literature, accessible in the public domain, regarding the microbiological safety of irradiated food. The pathogens *Listeria monocytogenes*, *E. coli* O157, *Salmonella* were targeted as being particularly important, although other microorganisms were also considered.

Methods: All relevant publications and unpublished data (where appropriate) were collated and evaluated in order to construct an objective review with unbiased, rationalized conclusions. A number of agencies were contacted (IAEA, WHO, FAO and FDA) to obtain current position papers and reports. A secure database was constructed. 2200 references dating from 1927 were collated, of which approximately 21.6% were related to microbiological safety (with the exception of review papers).

Results: There is no evidence that irradiation induces radiation resistance in human pathogens or changes the spoilage pattern of the food. It was clear, however, that the saAme microorganism behaves differently in different food commodities, even in very similar commodities such as different types of lettuce (*E. coli*). Different serovars of the same genus can display very different behaviour under the same conditions (*Salmonella*). *Listeria* spp. demonstrated increased resistance at low temperatures and under modified atmospheres. There were also differences in survival of the organism in different components of multicomponent foods. There are issues with irradiation-induced sensory changes. Although not a safety issue, the occurance of sensory changes does limit the dose of radiation to which any food commodity can be subjected. There is a lack of evidence regarding the effect of irradiation on protozoa or viruses.

Significance: The behaviour of microorganisms is influenced considerably by the matrix in which the irradiation takes place and the prevailing environmental conditions. Combination treatments can provide a solution to organoleptic changes, but thorough investigation of each combination must be made to assure product safety. It may not be possible to accurately predict behaviour without laboratory trials being performed. The extent to which all new commodities must be subject to these trials must be a decision based upon risk assessment.

T1-11 Agar Enhances Pediocin Production in Broth and Reduces Degradation in a Soy-seasoned Salmon Roe Food Model

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Introduction: For bacteriocins to be effective, sufficient amounts must be available to engage pathogens in the food matrix. Several complex methods of enhancing bacteriocin production and reducing biodegradation in the food matrix have been investigated.

Purpose: In this study we investigate the potential of a common gelling agent (agar) to enhance pediocin production and subsequently protect produced bacteriocins in a food model.

Methods: Liquid (MRS) and semi-solid media (MRS supplemented with 0.2% Agar) were fermented using a pediocin-producer (*Pedioccoccus pentosaceus* Iz3.13). Cell growth (plate counts), biomass accumulation (optical density measurements), acidification (pH values) and bacteriocin production (agar well method values against *L. monocytogenes* IID 580) were determined regularly for 36 h at 30°C (with agitation once every 30 min). Both liquid and semi-solid fermentates were freeze-dried and the anti-listerial potential (*L. monocytogenes* IID 578, IID581, ATCC 7644 at 5 log CFU/g) of the freeze-dried powders in a soy-seasoned salmon-roe food model was determined.

Results: In semi-solid broth cell growth, biomass accumulation, and acidification were significantly slower ($P < 0.05$) while maximum bacteriocin concentration was significantly higher (2 fold) than in liquid broth. In soy-seasoned salmon roe, fermentate from semi-solid broth (3000 AU/ml) inhibited growth of a *L. monocytogenes* cocktail for up to 6 days while inhibition by an equated amount (in terms of inhibitory activity) of freeze-dried powder from liquid broth (3000 AU/ml) lasted only 3 days at 12°C. It was concluded that semi-solid broths slow down cell growth and biomass production but enhance pediocin production per cell.

Significance: Addition of agar is a simple way to enhance pediocin production in broth. Agar subsequently improves pediocin effectiveness in soy-seasoned salmon roe by reducing bacteriocin degradation.

T1-12 Effect of Colony Numbers Selected from Plating Media on *Salmonella* Serogroup Detection from Naturally Contaminated Chicken Carcasses

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Introduction: Attribution studies are being undertaken to link *Salmonella* serotypes associated with human illness to food sources. However, studies have demonstrated that culture techniques often influence the sensitivity and specificity associated with bacterial recovery.

Purpose: This study evaluated the influence on serogroups of *Salmonella* through selection of one to three colonies per plate as affected by culture plating media.

Methods: Two replications were performed on commercial broiler carcasses (n = 26) directly after defeathering. Carcasses were individually bagged, transported on ice and rinsed with 100 ml of buffered peptone for 1 minute. One milliliter of rinsate was placed into both GN Hajna (GN) and Tetrathionate (TET) broths and incubated at 37°C for 24 h for GN broth or 48-h for TET broth prior to transferring 0.1 ml to Rappaport-vassiliadis (RV) media. RV tubes were incubated at 37°C for 24-h then streaked onto BG Sulfa (BGS) and XLT-4. Following 24-h incubation at 37°C, three presumptive colonies were selected from each of the four plates and numbered in order of selection (first, second or third), confirmed and serogrouped.

Results: Overall, 90% (BGS; 47/52) and 94% (XLT-4;49/52) of rinsates were positive. No difference in number of positives was observed when 1, 2, or 3 colonies per plate were picked. By carcass, picking a second colony from each plate produced one additional serogroup 16 times and two additional serogroups twice. Picking a third colony produced one additional serogroup nine more times compared to one or two colony picks and once yielded two additional serogroups.

Significance: Picking a single colony may underestimate the diversity of *Salmonella* serogroups within a sample and demonstrates the need to test multiple colonies to increase accuracy. Further characterization to the serotype level and on samples after chill will add additional information. Prior to use in an attribution study, limitations of culture methods/selection criteria for *Salmonella* should be established in both human and food laboratories.

T2-01 Inactivation of *Listeria monocytogenes* on Hams by Lauric Arginate Shortly after Vacuum-packaging

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Introduction: Strategies for control of *Listeria monocytogenes* on post-lethality exposed Ready-to-Eat meats may involve growth inhibition for the duration of the refrigerated product shelf life or bacteriocidal treatments during or shortly after the packaging stage of production. Lauric arginate (LAE), which has shown promise as a bacteriocidal treatment against *L. monocytogenes*, can be applied topically to thoroughly cover the surfaces of hams during the vacuum-packaging stage of production.

Purpose: This study measured and compared the short-term efficacy of LAE against *L. monocytogenes* present on various surfaces of large diameter hams.

Methods: Six different deli-style ham types with varied surface areas and characteristics were inoculated with *L. monocytogenes* at ca. 6.5 log CFU/ham and spray treated with between 15 and 27 ml of 9,090 ppm LAE solution or an equal volume of water (control) prior to vacuum-packaging. After 48-h at 4.4°C, populations were recovered from ham and interior packaging surfaces by use of a surface rinse method with DE neutralizing broth and were then plated on modified Oxford medium. Differences in populations on water- and LAE-treated hams were used to calculate log reductions for each treatment on a per-package basis.

Results: Log reductions of *L. monocytogenes* were > 2-log CFU/package on all ham types except one. Reductions on hams that had received a liquid smoke drenching during processing and prior to LAE treatment at 22 ml were lower than those on all other types, at 1.49 ± 0.60 log CFU/package, but reductions on hams with a very similar formulation that had been processed with a hot oil surface browning prior to LAE treatment at 22 ml were 2.20 ± 0.12 log CFU/package. Within each ham type, log reductions were not significantly different ($P > 0.05$) between treatment volumes spanning a 4-ml range.

Significance: This study demonstrated the efficacy of LAE against *L. monocytogenes* on several ham types, thereby validating LAE as a post-lethality treatment for inactivation of the pathogen.

T2-02 Short Term Bacteriocidal Efficacy of Lauric Arginate toward *Listeria monocytogenes* Present on the Surface of Frankfurters

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Introduction: Aqueous antimicrobials such as lauric arginate (LAE) have potential for controlling *Listeria monocytogenes* on Ready-to-Eat meats, but research is needed to better define optimal treatment conditions.

Purpose: This work was designed to elucidate the optimal conditions of post-lethality LAE application to frankfurters destined for vacuum-packaging in order to achieve > 1-log reduction of *L. monocytogenes*.

Methods: Frankfurters were inoculated with *L. monocytogenes* and placed in packages (8 in each package) prior to spray treating with between 2 and 4.5 ml of solutions of 5,000 or 8,000 ppm LAE, 5,000 ppm LAE plus liquid smoke flavor, or water (control). Inoculated and treated frankfurters were vacuum-sealed and stored at 4.4°C for 0, 2, 4 and 8 days before surfaces were analyzed for surviving *L. monocytogenes* by use of a rinse method and plating on modified Oxford medium.

Results: Treatment with 2 ml of 5,000 ppm LAE reduced *L. monocytogenes* from 7.13 to 5.82 log CFU/package on day 0, and 3 ml reduced populations from 7.22 to 5.79 log CFU/package on day 0. Treatment with LAE plus smoke flavor did not result in significantly different ($P < 0.05$) populations of *L. monocytogenes* compared to LAE alone. Log reductions on frankfurters 2 days after treatment with 3 to 4.5 ml of 5,000 ppm LAE solution ranged from 1.80 to 2.22, but were not significantly different between treatment volumes. All treatments resulted in reductions of ≥ 1.68-log CFU/package, and many of the treatments caused > 2-log reduction within 2 days, with no further declines evident at day 8.

Significance: This study demonstrates the short-term efficacy of LAE against *L. monocytogenes* on vacuum-packaged frankfurters and may provide supporting data for post-lethality treatments of frankfurters and sausages for control of the pathogen.

T2-03 DSC *In vitro* Inhibition of *Listeria monocytogenes* Exposed to Octanoic Acid and Acidic Calcium Sulfate Alone and in Combination

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Introduction: There is growing consensus that inhibition of foodborne pathogens can be improved by using combinations of antimicrobials on Ready-to-Eat (RTE) meat surfaces. Pairing different antimicrobials and utilizing their individual activities may be able to enhance the inhibition of pathogen growth.

Purpose: The purpose of the study was to evaluate the capability of Octa-Gone (Ecolab, St. Paul, MN; active agent: Octanoic Acid (OCT)) alone or in combination with Safe2O:RTE01 (Mionix, Round Rock, TX; active agent: Acidic Calcium Sulfate (ACS)) to inhibit the Gram-positive foodborne pathogen *Listeria monocytogenes in vitro*.

Methods: Pathogen inhibition was determined via a checkerboard assay. Strains of the pathogen (Scott A, 310, National Animal Disease Center (NADC) 2783 and 2045) were incubated aerobically in tryptose phosphate broth adjusted to pH 5.0. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial that resulted in < 0.05 change in optical density at 630 nm (OD630) after 24 h of incubation at 35°C. Following MIC determination, experimental strains were exposed to OCT and ACS in combination. Strains were exposed to MIC and sub-MIC levels of antimicrobials; inhibition was again defined as < 0.05 change in OD630 after 24 h of incubation. Fractional inhibitory concentration (FIC) index values were defined as the sum of the FIC of antimicrobial A and antimicrobial B. Antimicrobial FIC values were calculated as the MIC of antimicrobial A in combination (concentration of A that produced inhibition across all concentrations of antimicrobial B) divided by the MIC of antimicrobial A applied singly. Antimicrobial interactions were defined as synergistic, additive, or antagonistic when the FIC index was < 0.5, 0.5 to 2.0, or > 2.0, respectively.

Results: The MIC for OCT and ACS was 25.00 ppm active agent and 0.156% (v/v) for *L. monocytogenes* Scott A, 310, NADC 2783, and NADC 2045, respectively. FIC index values for the OCT + ACS combination were 1.5, 2.0, 1.0 and 1.5 for *L. monocytogenes* Scott A, 310, NADC 2783 and 2045, respectively.

Significance: Results indicate that the combination of the acidifying antimicrobials Octanoic Acid and Acidic Calcium Sulfate produces an additive-type inhibition upon *L. monocytogenes in vitro*. Further research is required to investigate and characterize the interactions of these and other approved antimicrobials for the inhibition of microbial pathogens on the surfaces of RTE meat products.

T2-04 DSC The Effect of Gaseous Ozone on the Survival of Surface Attached Environmental *Listeria monocytogenes* Serotype 1/2a

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Introduction: Gaseous ozone has considerable potential for use as a terminal sanitizer in the food industry. Traditionally used antimicrobials and fumigants have led to antimicrobial resistance and potential adverse health effects. Ozone leaves no toxic residues and has GRAS status for food processing. Listeriosis is an increasing problem in the over-60 age group and *Listeria monocytogenes* can be found on a range of surfaces within food processing plants and can

form biofilms that contain adhered bacteria that can be more resistant to disinfectants and sanitizers than planktonic organisms.

Purpose: To determine the effect of gaseous ozone on survival of surface attached *Listeria monocytogenes* isolated from the floor drains of a chilled ready to eat food processing plant.

Methods: Stainless steel coupons (25 cm^2) were inoculated with 0.1 ml of a suspension containing 10^9 CFU/ml of the environmental isolate. The inoculum was spread over the entire surface and allowed to dry. The coupons were exposed to gaseous ozone (2 to 45 ppm) for 1 hour. Coupons were oriented horizontally, vertically and inverted. Surviving organisms were recovered by swabbing and spread-plating on tryptone soya agar. Data were analyzed using a two-way ANOVA in Minitab 15.

Results: At lower ozone concentrations (2 to 5 ppm) there was no significant difference in survival of the organism between test and control coupons (0.4 log reduction). The results showed a significant difference ($P \leq 0.05$) between control and test coupons at both 10 and 45 ppm (up to 2.9 log reduction). There were no significant differences between different orientations of coupons treated (horizontal, vertical or inverted). The environmental isolate displayed increased resistance compared to culture collection strains of *Listeria* spp. (4 log reduction when exposed to 5 ppm for 1 h).

Significance: The reduction in survivors of surface attached *L. monocytogenes* by gaseous ozone is concentration dependent. It is not dependent on surface orientation. This is advantageous over traditional fogging, where vertical and inverted surfaces are less exposed to the fogging agent. It is hypothesized that the mechanism of resistance of this strain could be that this strain was isolated from a high-care food processing plant, where stringent validated cleaning protocols were practiced, potentially leading to enhanced resistance to antimicrobials.

T2-05 DSC Contrast in the Antibiotic-resistance Profiles of *Campylobacter* Isolates Originating from Different Poultry Production Facilities (Broiler Breeder Hens, Broilers, and Leghorn Hens) in the Same Geographical Region

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Introduction: Antimicrobial resistance of foodborne pathogens is of major concern from both human and animal health perspectives, and resistance profiles of *Campylobacter* spp. from individual poultry facilities have been extensively studied. However, a comparison of antimicrobial resistance profiles across different types of poultry facilities is lacking.

Purpose: This study was conducted to compare the antimicrobial resistance profiles of *Campylobacter* isolates originating from three different types of poultry production facilities.

Methods: Campylobacter isolates originating from commercial broiler breeder hens (n = 53), broilers (n = 103) and Leghorn hens (n = 114) in the same geographical area (within 60 miles) were analyzed. For speciation, a standard BAX® PCR method was used while susceptibility testing was performed using CLSI standards and recommended quality control organisms. Isolates were examined for susceptibility using a semi-automated broth microdilution system (Sensititre™) and nine antimicrobials: azithromycin, clindamycin, ciprofloxacin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline.

Results: From breeder hens, 49% of the isolates were *C. jejuni* and 51% *C. coli*. From broilers, 99% of the isolates were *C. jejuni* and 1% *C. coli*. From Leghorn hens, 46% of the isolates were *C. jejuni*, 53% *C. coli*, and 1% *C. lari*. All of the isolates were susceptible to azithromycin, clindamycin, gentamicin, and florfenicol. From broiler isolates, 61% were resistant to ciprofloxacin and nalidixic acid compared to 13% of the breeder hen isolates and none of the Leghorn hen isolates. Forty-five percent of the breeder hen isolates were resistant to erythromycin but none of the broiler or Leghorn hen isolates showed resistance. Resistance to tetracycline was most common across all three production facilities, with 59%, 42%, and 16% for broiler breeder, broiler, and leghorn isolates, respectively.

Significance: This study indicates that husbandry practices may influence antimicrobial resistance profiles of *Campylobacter* spp. isolated from different poultry production facilities.

T2-06 DSC Inactivation of Surface-attached and Tissue-infiltrated *Escherichia coli* O157:H7 on Lettuce and Spinach Using Allyl Isothiocyanate, Carvacrol and Cinnamaldehyde in Vapor Phase

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Introduction: The antimicrobial activity of essential oils and their constituent compounds is well documented. These antimicrobials in their vapor phase might be effective in inactivating *Escherichia coli* O157:H7 on leafy greens.

Purpose: We determined the ability of the vapor of allyl isothiocyanate (AIT), cinnamaldehyde and carvacrol to inactivate surface-attached and tissue-infiltrated *E. coli* O157:H7 on lettuce and spinach.

Methods: GFP-tagged *E. coli* cocktail was surface inoculated or vacuum infiltrated within individual lettuce and spinach leaves. The samples were treated with various concentrations of the antimicrobials in the vapor phase at 0, 4, and 10oC in an enclosed container. The log reduction of the pathogen on the treated samples was statistically compared to that on untreated positive control. Pathogen infiltration and inactivation with the tissue were observed under confocal scanning laser microscopy.

Results: On lettuce surface, the vapor of the lowest concentration of these antimicrobials inactivated > 4 log of *E. coli* O157:H7 at 0 and 4°C in 4 days and at 10°C in 2 days. However, within lettuce tissue, the highest concentration reduced the pathogen population by 4 log at 0°C and 2 to 4 log at 4°C in 4 days. These concentrations also reduced the population by 1 to 3 log units at 10°C in 2 days. The reduction of the population on spinach surface was 1 log less than the reduction observed on lettuce surface. Reduction of the population on spinach tissue was 2 and 3 log less than within lettuce tissue at 0 and 4°C, respectively. Overall, greater inactivation occurred on lettuce than on spinach leaves and on the leaf surfaces than within tissue.

Significance: Using antimicrobials in the vapor phase may improve the safety of refrigerated leafy greens marketed in sealed packages.

T2-07 Withdrawn

T2-08 SaniTwice™: A Hand Hygiene Solution for Food Handlers when Water is Unavailable

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Introduction: The FDA Food Code specifies that food handlers must maintain clean hands by washing with soap and water. In special cases where handwashing sinks are not available, employees may use chemically treated towelettes for handwashing; however, there is limited data to demonstrate the antimicrobial efficacy of such a procedure. Because food is frequently prepared and served in remote areas where water and/or sinks are unavailable (e.g., catering in remote locations), an effective hand hygiene option for these environments is needed.

Purpose: Evaluate the antibacterial efficacy of a new two-step hand hygiene procedure [SaniTwice], using an alcohol based hand sanitizer (ABHS) to determine whether it is an appropriate option for food handlers when water and/or sinks are unavailable.

Methods: A modification of ASTM E1174 ("Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations") was used. Hands were contaminated with approximately 10^9 *Escherichia coli* suspended in beef broth to replicate light to moderate soils found commonly in food service settings. Ten participants used each of the following three test configurations: a handwash with non-antimicrobial soap, the SaniTwice procedure, and a modified SaniTwice procedure. Briefly, the SaniTwice procedure involved "washing" with an excess of ABHS and paper towel drying, followed by reapplication of ABHS according to label instructions. The modified SaniTwice method was similar, but an alcohol-based antimicrobial hand wipe was used in the first step. Log reductions from baseline were calculated for each configuration.

Results: The non-antimicrobial handwash, SaniTwice, and modified SaniTwice configurations achieved $\log_{10}$ reductions of 2.92 ± 0.58, 2.83 ± 0.66 and 2.63 ± 0.60, respectively. All test configurations were statistically equivalent by ANOVA ($P > 0.5$).

Significance: The SaniTwice method should be considered an effective option for hand hygiene when water or handwashing facilities are not available, because it achieved bacterial reductions equivalent to traditional hand washing. This can result in a feasible, practical hand hygiene intervention for remote food service situations.

T2-09 Interaction between Histamine-producing Bacteria and Prediction of Biogenic Amine Formation in Seafood

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Introduction: Morganella morganii, M. psychrotolerans and *Photobacterium phosphoreum* are strongly histamine producing bacteria. They have all been responsible for biogenic amine formation in specific fish products that caused histamine fish poisoning. Growth and histamine formation by individual cultures of *M. morganii* and *M. psychrotolerans* can be predicted by available mathematical models. It remains a challenge to predict biogenic amine formation by co-cultures of these bacteria and this is important for the evaluation of seafood safety.

Purpose: To predict growth and histamine formation for co-cultures of *M. morganii, M. psychrotolerans* and *P. phosphoreum* at constant and dynamic storage temperatures.

Methods: Growth and histamine formation by *M. morganii. M. psychrotolerans* and *P. phosphoreum* were studied in broth with added amino acids for individual (n = 8) and co-cultures (n = 16) of the three species. Constant and changing storage temperatures between 5°C and 25°C were evaluated. Growth was measured by viable counting for *Morganella* cultures and by a conductance based incubation method for *P. phosphoreum*. Biogenic amines were determined by HPLC. Root mean square error (RMSE) between observed and predicted growth curves (log CFU/ml) was used to evaluate the inhibitory effect of microbial interaction.

Results: High concentrations of *M. morganii* prevented growth of *M. psychrotolerans* and *P. phosphoreum*. High concentrations of *M. psychrotolerans* had the same inhibitory effect on *M. morganii* and *P. phosphoreum*. For each set of growth curves the effect of interaction was appropriately described by the differential form of an expanded Logistic growth model. The interaction term reduced RMSE from 2.0 ± 0.4 to 0.8 ± 0.5 log CFU/ml. Histamine formation by individual and co-cultures of *Morganella* was well predicted by combining the growth models for each species with a constant yield factor of -8.0 ± 0.44 log (mg histamine/CFU). High concentrations of *P. phosphoreum* produced several biogenic amines and reduced growth rates of *M. morganii* and *M. psychrotolerans* by 38 to 52% but did not prevent their growth.

Significance: These results are important for prediction of histamine formation in seafood, particularly at variable storage temperatures at which both psychrotolerant and mesophilic bacteria contribute to biogenic amine production.

T2-10 DSC Use of Edible Coatings Containing Organic Salts to Control *Listeria monocytogenes* on Cold-smoked Salmon Slices and Fillets

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Introduction: The high incidence of *Listeria monocytogenes* (6 to 36%) in cold smoked salmon (CSS) is of concern, as CSS is a Ready-to-Eat product. No post-processing measures are currently available to control this pathogen in CSS.

Purpose: The objective of this study was to develop an effective antimicrobial edible coating containing organic salts to control the growth of *L. monocytogenes* in CSS slices and fillets.

Methods: Individual or binary combinations of sodium lactate (SL, 1.2 and 2.4%) and sodium diacetate (SD, 0.125 and 0.25%) as well as 2.5% OptiForm (commercial formulation of SL and SD) were incorporated into five edible coatings: alginate, κ-carrageenan, pectin, gelatin or starch. The coatings were applied onto the surface of CSS slices inoculated with *L. monocytogenes* (~500 CFU/cm^2) and stored at 22°C for 6 days. In the second phase of the study, CSS slices and fillets inoculated with the pathogen (~500 CFU/cm2) were coated with alginate-based coatings into which were incorporated single or binary combinations of organic salts and OptiForm and then stored for 30 days at 4°C.

Results: Alginate coating, the most effective carrier when supplemented with OptiForm, suppressed the growth of *L. monocytogenes* in CSS slices. By the end of the ambient temperature storage, the counts on treated samples had decreased to 2.4 log CFU/cm^2 while the populations of *L. monocytogenes* on control samples had increased to ~ 7.6 log CFU/cm^2. During refrigerated storage, alginate coatings incorporating OptiForm delayed the growth of *L. monocytogenes* on CSS slices and fillets with final counts reaching 2.5 and 3.7 log CFU/cm^2 respectively, while the populations in their untreated counterparts were significantly higher ($P < 0.05$) reaching 6.6 and 6.1 log CFU/cm^2 respectively.

Significance: This study demonstrates the antilisterial effectiveness of using an alginate-based edible coating containing antimicrobials to enhance the microbiological safety of filleted and sliced smoked salmon.

T2-11 Science-based Retail Food Process Development

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Introduction: Chefs and food scientists are responsible for product development and assuring the safety of new processes. The kitchen is the place where the cook applies a procedure, if necessary, to reduce the hazards in food from the farm to meet a Food Safety Objective (FSO) such as < 1 *Salmonella* in 25 g in the finished food. The Food Code says that the retail food process developer can use any innovative approach, providing the process shows compliance with federal performance standards and is documented in a validated HACCP plan.

Purpose: This presentation describes the science base for the use of the HACCP design method to develop new and innovative processes in retail operations.

Methods: There are five basic food processes in retail, following USDA HACCP. I. Raw, not heat treated II. Not fully cooked, with inhibitors to make shelf stable III. Fully cooked, not shelf stable IV. Fully cooked, with inhibitors to make shelf-stable V. Commercially sterile, shelf-stable process development starts with a detailed flow chart followed by the recipe with all ingredients and process steps. These documents are used to do the validation study and for HACCP plan process documentation, regardless of what process is being validated – cleaning food contact surfaces, washing vegetables, pasteurizing food, fermenting food, etc. The equation for process design comes from the ICMCF: $H_o + \sum I - \sum P \rightarrow \leq FSO$. H_o is the hazard coming into the process. The FSO is the level of hazard that can be in the final product when consumed. $+\sum I$ is the increase in the hazard before consumption, such as growth of a pathogen in chicken salad on a buffet line before it is consumed or thrown out. $-\sum R$ is the prevention, elimination or reduction control that the cook/process engineer designs into the process to assure that customer health is protected (e.g., discard salad after 6 h if held at 50°F for <1 log increase in *Listeria monocytogenes*).

Results: This HACCP-based retail process development has been used with large chain operations and individual kitchens. It is a part of the Active Managerial Control process, resulting in new processes that are completely transparent and for which, in the past seven years, there has been an undetectable level of failures.

Significance: Retail process and product development is critical to a dynamic, profitable retail food industry. HACCP design assures that new processes provide adequate consumer protection.

T2-12 Incidence of Melamine in Milk Powder and Infant Formula Sold on the East African Market

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Introduction: Melamine is an organic base with the chemical formula $C_3H_6N_6$ and named of 1,3,5-triazine -2,4,6-triamine. A large melamine contamination event of milk-containing products, including infant formula, was reported from China. At least 22 dairy manufacturers across the country were found to have melamine in their products (levels varied between 0.09 mg/kg and 2.563 mg/kg). According to China's Ministry of Health, some 294,000 children had received hospital treatment and six babies with kidney stones have died.

Purpose: The present study aims to determine (1) the incidence of melamine in milk powder and infant formula sold in Dar es Salaam (Tanzania, East Africa) and (2) the incidence of illegal, none labelled milk powder of Chinese or unknown origin on the local informal (black) market.

Methods: Melamine determination was carried out using the AgraQuant® Melamine Sensitive Assay which is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level of melamine and is intended for use in milk, milk powder and other dairy products (Romer Labs®, Singapore Pte Ltd.). The ELISA test kit has a quantification range of 0.5 to 25.0 ppm. A total of 49 milk powder samples were randomly selected in Dar es Salaam, which is one of the biggest seaports on the East African Coast and a centre of international trade, both legal and illegal.

Results: 26/49 (53.1%) samples were market brands and 8/26 (30.7%) were clearly labelled as being intended for infants. In spite of the national import prohibition, 23 samples (46.9%) were illegally sold on the local informal market or black market. These samples were not labelled at all and might be of Chinese origin. In 44 of 49 samples, melamine could not be detected at all. However, 3 of 49 (6.1%) samples showed a melamine concentration between 0.6 to 5.7 ppm. All melamine positive samples were international market brands. In the meantime, the Tanzanian Food and Drug Regulatory Authority could confiscate more than 43.89 tons of milk products imported from China. The analysis of these samples will start now.

Significance: This is the first study proving the incidence of melamine in milk powder and infant formula sold in East Africa. This circumstance is so much the worse, taking into consideration that milk powder is used as food aid for millions of African people, especially malnourished infants and young children or people living with HIV or AIDS.

T3-01 Comparison of Different Pre-enrichment Strategies for the Recovery of *Salmonella* from Internally Contaminated Red Round Tomatoes

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Introduction: In the past decade, there have been 13 tomato-related *Salmonella* outbreaks in the U.S. that resulted in thousands of foodborne illnesses and multiple deaths. Internalization of foodborne pathogens in fresh produce is of particular concern to the food safety community.

Purpose: Comparison of two alternative pre-enrichment procedures (quartered and stomached) against the *Bacteriological Analytical Manual* (BAM) *Salmonella* culture method (whole soak) for the detection and isolation of *Salmonella* from internally contaminated red round tomatoes.

Methods: Red round tomatoes were purchased from a local grocery store. Tomatoes were inoculated by injecting 50 µL of one of two *Salmonella* serovars (e.g. *S.* Saintpaul and *S.* Weltevreden) in the stem scar or side perpendicular to the stem scar. All tomatoes were disinfected twice with ethanol, allowed to air dry overnight, and held for 4 days at 4–6°C following inoculation. Twenty tomatoes each were quartered, stomached, and soaked (total of 120 analyzed). Quartered and stomached tomatoes were pre-enriched in Universal Preenrichment at a 1:1 sample-to-broth ratio. Soaked (whole) tomatoes were pre-enriched at a 1:1.5 sample-to-broth ratio. Samples were incubated for 24 h at 35°C. The BAM *Salmonella* culture method was followed thereafter (tomatoes were treated as a low microbial load food).

Results: Findings from tomatoes injected in the stem and side indicate that the alternative methods of quartering (79 positive) and stomaching (80 positive) were more effective than the BAM soak method (59 positive) for the recovery of *Salmonella* from internally contaminated tomatoes.

Significance: This study found that the alternative pre-enrichment methods of quartering and stomaching were equivalent to one another and superior to the current BAM *Salmonella* culture method. These results address the need to develop improved methodology for the detection of internalized *Salmonella* in tomatoes.

T3-02 DSC Rapid Detection of *Salmonella* Typhimurium from Spiked Lettuce and Tomatoes Using Real-time Reverse Transcriptase-polymerase Chain Reaction

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Introduction: Recent outbreaks of *Salmonella* linked to fresh produce emphasize the need for rapid and sensitive assays to help control outbreaks. Reverse-transcriptase PCR (RT-PCR) detects the presence of mRNA (shorter half-life than DNA), with greater potential of detecting viable cells. Rapid real-time methods using fluorescent dyes and probes simultaneously detect and confirm the presence of target nucleic acid, eliminating the need for gel electrophoresis.

Purpose: The objective of this research was to rapidly detect *Salmonella Typhimurium* from spiked lettuce and tomatoes using real-time RT-PCR.

Methods: Washed and ultraviolet light treated lettuce (~25 g) and tomato (~100 g) samples were inoculated with high (10^8 to 10^6 CFU) and low (10^3 to 10^1 CFU) *Salmonella* Typhimurium overnight cultures. Samples were then rinsed or hand massaged with 225 ml 0.05 M glycine-saline buffer containing 0.05% Tween and 3% beef extract. Un-inoculated washed produce and sterile buffer were used as negative controls; with *S.* Typhimurium as a positive control. RNA was extracted from each sample, using the Qiagen RNeasy Mini Kit. RT-PCR was carried out using a SYBR Green I RT-PCR kit with previously described *Salmonella*-specific invA gene primers and an internal amplification control (IAC) to eliminate false negatives. Reaction conditions were RT at 50°C/30 min; PCR at 95°C/45 s, 58°C/45 s, 72°C/45 s for 40 cycles followed by melt temperature™ analysis in a BioRad iCycler to determine specific invA product (Tm = 87.5°C) and IAC (Tm=82°C). To improve detection sensitivity of low inocula, spiked lettuce and tomatoes were pre-enriched in buffered peptone water for 8 h at 37°C, followed by RNA extraction and RT-PCR detection. Each experiment was repeated twice.

Results: Real-time RT-PCR after 8-h pre-enrichment, Qiagen RNA extraction and the SYBR Green I kit gave *Salmonella* detection up to 10^3 CFU from lettuce and 10^4 CFU from tomatoes. Without enrichment, detection limits were 10^6 CFU for lettuce and 10^7 CFU for tomatoes. Sensitive and rapid detection of *Salmonella* from spiked lettuce and tomatoes could still be obtained within one day (~ 2 working shifts).

Significance: This assay shows tremendous potential for the sensitive and rapid detection of *Salmonella* from lettuce and tomatoes within 24 h for routine testing and real-world scenarios.

T3-03 Development of Quantitative Real-time PCR Method for the Detection and Characterization of Toxigenic *Clostridium difficile*

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Introduction: The increased prevalence of *Clostridium difficile* associated diarrhea (CDAD) in humans and the recent detection of the pathogen in retail meat products has necessitated the development of rapid detection methods. The pathogenicity locus (PaLoc) codes two toxins, enterotoxin A (tcdA) and cytotoxin B (tcdB), which are responsible for the majority of CDAD cases. It is important to develop a method that will target these toxins genes for detecting toxigenic strains of *C. difficile*.

Purpose: The main objective of this study was to develop a quantitative real-time PCR-based detection method for the rapid and sensitive detection of toxigenic *C. difficile* in food animals and retail meat products.

Methods: Primers and dual labeled Taqman probes were designed specifically targeting an internal control topoisomerase gene (tpi), and tcdB gene coding for toxin B. Amplification was performed using purified DNA templates of *C. difficile* isolates with previously characterized toxin profiles. Quantification analysis and sensitivity of the method was measured by performing single- and multiplex reactions to generate standard curves using serially diluted purified plasmids containing each target.

Results: We were able to correctly identify and detect *C. difficile* under multiplex conditions using the tpi and toxB probes. The detection threshold of the multiplex real-time PCR was 10 copies/reaction. We detected high correlation between single and multiplex reactions (r_2 >0.96). The real time assay developed in this study had a very high sensitivity of 100% (20/20) for tpi and 91% (10/11) for tcdB genes. The overall specificity was 100% for both genes. The positive predictive power of the assay was 100% for both genes (20/20 and 10/10, respectively) while the negative predictive power was 100% for tpi (6/6) and 94% for tcdB (15/16). Overall, we have developed a method that will allow us to rapidly detect and quantitate *C. difficile* in different sources.

Significance: The ability to quickly identify and assess the toxingenicity *C. difficile* using the above method is invaluable both for the rapid detection in food animals and retail meats as well as in the diagnosis of CDAD in humans.

T3-04 ISO 16140 Validation of a Real-time PCR Method for the Simultaneous Detection of *Escherichia coli* O157:H7 and *Salmonella* spp. in Beef in Ten Hours

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Introduction: The meat industry routinely releases products having a short shelf life (< 7 days), requiring very rapid analytical methods for safety control before consumers can get them in their plates. Because traditional bacteriological methods and most of the alternative methods cannot deliver analytical results in less than 24 to 48-h , we developed the GeneDisc DUO *E. coli* O157 and *Salmonella* spp. allowing the detection of both organisms in less than 10 h for raw ground beef products. The performance of this method had to be demonstrated through official validation schemes before it could be used for routine analysis by the food industry.

Purpose: Two innovative multiplex PCR assays were developed and combined for the routine detection of *Salmonella* spp. and *E. coli* O157 STEC and the identification of EHEC in meat. This method was validated according to the ISO 16140 standard and was compared to ISO 6579 and ISO 16654 for *Salmonella* spp. and *E. coli* O157, respectively.

Methods: Briefly, 25 g of food sample were enriched in BPW for 8 h at 41.5°C or for 16 h at 37°C. Bacterial DNA was extracted from the enrichment broth, then analyzed with a PCR-based technology allowing the amplification of specific sequences for (i) *Salmonella* spp (iroB), (ii) *E. coli* O157 (rfbE), (iii) stx genes, and (iv) eae gene. When the rfbE PCR and/or STEC PCR was (were) positive, the DNA extract was also further analyzed using a second PCR assay targeting the presence of the *flagella* H7 (flicH7) and of the main *E. coli* serovars (O26, O103, O111 and O145) that have been associated with HUS in humans.

Results: Accuracies, specificities and sensitivities were evaluated for 70 meat samples. Results obtained using the Standard and GeneSystems methods were shown to be equivalent ($\alpha = 0.05$). The limits of detection of PCR methods were 0.1 to 1.0 and 0.1 to 1.2 CFU/25 g for *Salmonella* and *E. coli* O157:H7, respectively. Finally, 50 target strains and 32 non-target strains were analysed for the specificity study. The second part of the validation was a ring trial (12 independent laboratories) using ISO 16140 guidelines. Only three discordant assays were noticed amongst a total of 576 analyses. The variability of the PCR and ISO methods were shown to be equivalent.

Significance: This multiplex PCR method was AFNOR approved, as it showed results equivalent to the Standard methods. It is an efficient tool, offering fast and reliable results for the routine onsite control of fresh meat products. In addition, the combination of multiparametric assays allowed the discrimination of pathogenic O157 using the detection of *stx* and *eae* virulence genes simultaneously to O157.

T3-05 Independent Laboratory Evaluation of a Real-time PCR Test for Detection of *Listeria* spp. in Selected Foods from a Single Primary Enrichment

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Introduction: Listeria spp. are found frequently in the food-processing environment and have been isolated from a variety of food products. Testing methods are available for the detection *Listeria* in foods and environmental surfaces, which take 2 days for a presumptive result. R.A.P.I.D.® LT Food Security System for *Listeria* is a rapid detection method which couples real-time PCR with a short enrichment time. The test method allows for the detection of a presumptive result from a food sample or environmental surface in less than 24 h, utilizing a single primary enrichment followed by detection using real-time PCR.

Purpose: The objective of this study was to compare the performance of the test method, using either enrichments either alone or with pooling of multiple sample enrichments into one test sample, to that of established USDA reference methods for deli turkey and stainless steel surfaces.

Methods: For deli turkey, twenty 25 g samples inoculated at < 1 CFU/g and 25 uninoculated control samples were analyzed, using the test method with and without pooling and by the appropriate reference method. In addition, a 3-tube MPN analysis was performed in order to determine the level of *Listeria* present in the deli turkey. For stainless steel coupons, twenty 25 g sponges samples taken from surfaces inoculated at < 1 CFU/g and 25 uninoculated control samples were analyzed, using the test method with and without pooling and by the appropriate reference method.

Results: A Chi square analysis according to Mantel-Haenszel for unpaired samples revealed no significant difference in the number of confirmed positive stainless steel samples. A significantly higher number of confirmed positive deli turkey samples was observed between the test method, with or without pooling, and the USDA method.

Significance: The real-time PCR method could be used to detect the presence of *Listeria* in deli turkey and stainless steel in less time than is required by the traditional reference method.

T3-06 Statistical Data Analysis of Real-time PCR Results Derived from Single Copy Amplification
DSC

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Introduction: The validation of real-time PCR systems and above all the proof of the detection limit of this method is a frequently and intensively discussed topic. We present a statistical method for the accurate determination of DNA amounts < 10 target molecules by use of real-time PCR. The implication of this method is the possibility of distinct validation of real-time PCR assays and the generation of absolute DNA standards needed for quantification with this enzymatic method in routine diagnostics.

Purpose: The purpose of this study was to evaluate a novel validation tool for real-time PCR assays based on the theoretical possibility of the amplification of one single DNA target. The ability to detect such low DNA target concentrations reliably by real-time PCR should be clearly demonstrated. Consequently a validation method based on these prerequisites that allows the absolute evaluation of real-time PCR assays to be established.

Methods: Real-time PCR was carried out by targeting a 274 bp fragment of the prfA gene of *L. monocytogenes*. Fit of the empirical data to the theoretical predictions was tested with the Kolomogorow – Smirnov (K-S) test using the SPSS 14.0 statistical software package.

Results: The ability of the prfA real-time PCR assay to detect reliably one target molecule could be clearly demonstrated (pavg. = 0.52). The coherence of the results of samples containing < 10 target molecules and samples containing DNA amounts within the range of fluorescent measurement could be clearly demonstrated. The evidence for the accuracy of the newly developed validation method was shown both statistically and directly. The explicit determination of assays with a detection limit of one copy and assays with such a limit of three copies is exemplarily demonstrated. We also demonstrate that real-time PCR at best starts from the first cycle with a certain efficiency and proceeds with this efficiency until saturation of the reaction.

Significance: The results show that an absolute validation of real-time PCR assays is possible. The Ct - values of certain initial target amounts are fixed in dependence of the efficiency of the reaction. An absolute determination of DNA amounts is possible independent of conventional measurement methods. The validation tool also allows on-line monitoring of real-time PCR results in routine diagnosis.

T3-07 Evaluation of Multiple-locus Variable Number of Tandem Repeat Analysis (MLVA) to Subtype *Listeria monocytogenes* Directly in Food Samples

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Introduction: Listeria monocytogenes is responsible for severe and fatal infections in humans. *Listeria* contamination occurs quite often in a wide range of foods due to its ubiquitous nature. Isolates need to be characterized to a strain level in order to accurately diagnose their infection, to understand the epidemiology, to investigate outbreaks, and to effectively prevent and minimize foodborne listeriosis.

Purpose: The purpose of this research was to evaluate the multiple-locus variable number of tandem repeat analysis (MLVA) for sub-typing *L. monocytogenes* isolates in pure cultures and in food matrices.

Methods: DNA was amplified in two multiplex PCR assays using FAM-labeled forward primers based on six specific loci. The PCR fragments were separated using an ABI 3730 Genetic Analyzer. The fragment data were analyzed using the GeneMapper and the BioNumerics software programs to determine the relationship of different isolates.

Results: The MLVA method resulted in 34 unique DNA fingerprint patterns from 46 *L. monocytogenes* isolates of 10 serotypes which had 34 AFLP patterns. The MLVA patterns of the 46 isolates remained unchanged in the presence of pre-enriched food matrices, including sausage, ham, chicken, milk and lettuce. The MLVA method successfully typed *L. monocytogenes* strains spiked in cheese and vegetable samples after 48-h enrichment at the initial inoculation levels of 1 to 5 CFU per 25 g of food or higher. The limits of detection (typing) of the MLVA method were 1,000 to 10,000 CFU per mL of pre-enriched food broth when evaluated using post-spiked sausage, ham, chicken, milk and lettuce samples.

Significance: The MLVA method was simple, highly discriminatory, and easy to perform with portable results. To our knowledge, this is the first report that describes the application of the MLVA method directly to food samples and demonstrates the possibility to obtain rapid and accurate subtyping results before a pure culture is isolated.

T3-08 A Real-time Loop-mediated Isothermal Amplification Assay for the Detection and Quantification of *Vibrio vulnificus*
DSC

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Introduction: Vibrio vulnificus, a Gram-negative halophilic bacterium that inhabits warm coastal and estuarine waters worldwide, is a significant cause of seafood-related deaths in the United States. Rapid and sensitive detection assays are needed to facilitate regulatory and industry personnel to better control potential risks of *V. vulnificus* infections from

seafood. Recently, a loop-mediated isothermal amplification (LAMP) method has been adopted by two research groups for use in detecting *V. vulnificus*.

Purpose: This study aimed to evaluate the two published LAMP assays and further develop a real-time LAMP assay for the detection and quantification of *V. vulnificus*.

Methods: A real-time platform was coupled with the two LAMP assays for real-time detection and quantification of *V. vulnificus*. Sensitivity and specificity of the two LAMP assays were evaluated by use of a collection of 20 *V. vulnificus* and 30 other bacteria.

Results: Both LAMP assays possessed good specificity, with no false positive or false negative results. One LAMP assay was found to be 10 times more sensitive than the other, with a lower limit of detection of approximately 24 *V. vulnificus* cells. When a real-time platform was used, both assays were able to detect positive samples within 1 hour of the reaction initiation.

Significance: The real-time LAMP assay developed in the present study could be adopted to detect and quantify *V. vulnificus* with high sensitivity and specificity.

T3-09 Inactivation of Shiga Toxin from *Escherichia coli* O157:H7 by Food-compatible Plant Compounds

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Introduction: Numerous foodborne diseases result from ingesting foods that are contaminated with bacterial toxins. *Escherichia coli* O157:H7 is a leading cause of foodborne illness. This human pathogen produces Shiga toxins (*stx*1 and *Stx*2), which inhibit protein synthesis by inactivating ribosome function.

Purpose: As part of an effort to develop food-compatible conditions to alter the activity of *stx*, we developed a novel and simple fluorescence cell-based assay for the detection of Stx and for identifying natural plant compounds for anti-*stx* activities. To establish our Vero-d2EGFP assay as a useful tool for the identification of toxin inhibitors, we screened a panel of natural plant compounds for anti-toxin activities.

Methods: A Vero cell line harboring a destabilized variant ($t_{1/2}$ = 2 h) of the enhanced green fluorescent protein (d2EGFP) was used to monitor the toxin-induced inhibition of protein synthesis. This Vero-d2EGFP cell line produced a fluorescent signal which could be detected by microscopy or with a plate reader.

Results: Dose response curves demonstrated that the *stx*2-induced inhibition of EGFP fluorescence mirrored the *stx*2-induced inhibition of overall protein synthesis and established a picogram/ml threshold for toxin detection. Although a number of food-compatible compounds such as tea catechins, carvacrol, and N-acetylcysteine were not effective inhibitors of *stx* activity under the test conditions, we discovered that grape seed or grape pomace extracts provided strong cellular protection against *stx*. The anti-toxin properties of the grape extracts were confirmed with an independent toxicity assay that monitored the overall level of protein synthesis in cells treated with purified *stx*2.

Significance: These results indicate that the Vero-d2EGFP fluorescence assay is an accurate and sensitive method to detect *stx*2 activity and can be utilized to identify toxin inhibitors. Our findings have also demonstrated that food-compatible and safe compounds with anti-toxin properties could be used to develop conditions for in vitro inactivation of toxins in food and have set a foundation for futures studies on the use of natural plant compounds against other bacterial toxins with mechanisms similar to *stx*.

T3-10 Effect of Pre-treatment and Post-treatment of Centrifugal Ultrafiltration Device on the Recovery of F-RNA Coliphage MS2

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Introduction: Most human pathogenic enteric viruses cannot be cultured, so they must be detected by molecular techniques. Male specific (F+) RNA coliphages, a potential surrogate for human enteric viruses, can be detected by culture and molecular assays, but numbers in contaminated food or water may be too low for direct detection. Ultrafiltration is a general concentration method for all virus types but there is little information on the recovery efficiency of F-RNA coliphages and enteric viruses.

Purpose: The objective was to optimize the recovery of concentrated F-RNA coliphage MS2 from Microsep 100K ultrafiltration devices, because the recovery of MS2 in initial trials was only 26 ± 8% by plaque assay.

Methods: Microsep 100K devices were washed with sterile water or treated with bovine serum albumin, polyethylene glycol or glycerol before the addition of MS2 suspensions and rinsed or sonicated after ultrafiltration. MS2 suspensions and concentrates were enumerated by plaque assay or by real time RT-PCR. The % recovery was determined and each data set for each treatment was tested for normal distribution and values for the means were separated by the Tukey test of the general linear model procedure.

Results: The mean recovery of MS2, as determined by plaque assay, increased significantly to 68 ± 20% or 85 ± 10% when ultrafiltration devices were treated with 1% BSA before concentration followed by rinsing or sonication, respectively, after concentration. Recovery of MS2 by sonication was significantly higher than by rinsing. The recovery of genome copies of MS2 by realtime RT-PCR from sonicated ultrafiltration devices pre-treated with 1% BSA was 65 ± 22%.

Significance: To our knowledge, this is the first study to use sonication as a post-treatment step to increase recovery of viruses from ultrafiltration devices. This optimized method for MS2 may have applications for concentrating a wide range of other viruses with an ultrafiltration device.

T3-11 Evaluation of VIDAS® Recombinant Phage Protein Technology for Detection of *Escherichia coli* O157:H7 in Produce and Spent Irrigation Water

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Introduction: With the recent recalls of spinach and leafy greens, there is an increased demand for testing for *Escherichia coli* O157:H7 in leafy greens and irrigation water. Due to the short shelf life of these products, a reliable method is needed to detect *E. coli* O157:H7 from leafy greens in less than a day. The VIDAS® E. coli O157:H7 Phage technology (ECPT) test is an automated enzyme-linked fluorescent assay (ELFA) using recombinant phage protein technology for the specific detection of *E. coli* O157:H7. The test method allows for same day detection of a presumptive result from leafy greens or spent irrigation water following 8-h enrichment in a proprietary BPW with antibiotics.

Purpose: The objective of this study was to compare the performance of the test method to that of established FDA BAM reference methods for lettuce, spinach, and spent irrigation water.

Methods: Lettuce, spinach and spent irrigation water were screened with the FDA BAM method for indigenous *E. coli* O157:H7 prior to inoculation. For each food type, twenty 25 g samples inoculated with < 1 CFU/g and 5 uninoculated control samples were tested for *E. coli* O157:H7, using the test method and the reference method. In addition, a 3-tube MPN analysis was performed in order to determine the level of *E. coli* O157:H7 present in the inoculated food.

Results: Using a Chi square analysis for unpaired samples at the 95% probability level, the rapid alternative method was found to be statistically equivalent to the reference method.

Significance: In this study, the alternative method detected the presence of *E. coli* O157:H7 in leafy greens and irrigation water in significantly less time than that required by the traditional reference method.

T3-12 Phenotype Independent Target Concentration for Detection of Pathogenic Bacteria in Meats

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Introduction: Faster time to result for pathogen detection requires concentration of target organisms. Phenotype dependent concentration methods targeting cell surface molecules (i.e. antibody and phage based methods) are subject to inherent failure modes. For example, among pathogenic *E. coli*, rough isolates (which constitute approximately 2% of clinical cases) do not express the O antigen target required for specific concentration by antibody or phage reagents, while for *Salmonella*, antibodies often react poorly with a subset of serotypes, leading to loss of sensitivity for detection of these strains.

Purpose: To overcome these inherent difficulties and still provide a shortened time to result for detection assays, we developed a method to concentrate target organisms independent of the expression of surface antigen.

Methods: E. coli O157:H7: Analysis of this method's efficacy for detection of *E. coli* O157:H7 in 375 g beef trim samples was performed, using samples spiked at a fractional positive level (1.6 CFU/375 g) The spiked samples were cold stressed, enriched for 6.5 h, and then tested both as individual enrichments and in a wet composite format, with four unspiked enrichments using the phenotype independent sample prep (PHISP) method prior to PCR analysis. The enrichments were incubated further to 24 h and tested directly by PCR. *Salmonella*: Analysis of PHISP efficacy for detection of *Salmonella* in 125 g ground pork samples was performed using samples spiked with *S.* Dublin at a fractional positive level (1.2 CFU/125 g) The spiked samples were cold stressed, enriched for 8 h and then were tested as individual enrichments using the PHISP method prior to PCR analysis. The enrichments were incubated further to 12 h and tested directly by PCR.

Results: E. coli O157:H7 beef trim enrichments: At 6.5 h 17 of 20 spiked and 0 of 5 unspiked enrichments were positive using both the single enrichment and wet composite sample prep methods prior to PCR detection. This was identical to the 24-hour enrichment results using direct testing of the enrichment. *Salmonella* ground pork enrichments: At 8 h enrichment 17 of 19 spiked and 0 of 4 unspiked samples were positive using the sample prep method prior to PCR analysis. This was identical to the end point direct testing of the enrichment.

Significance: This phenotype-independent method allows for target concentration of pathogens, with subsequent decreased time to result while avoiding the inherent failure modes associated with surface antigen capture methodologies.

T4-01 Comparison of Assurance GDS for *Listeria monocytogenes* and Assurance GDS for *Listeria* spp. Assays with Culture Methods for the Detection of *Listeria* in Selected Foods and Environmental Surfaces

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Introduction: The Assurance GDS™ for *Listeria* spp. and the Assurance GDS™ for *Listeria monocytogenes* assays incorporate novel immunomagnetic separation procedures and proprietary probe-based molecular detection technologies enabling accurate detection of either *Listeria* spp. or *Listeria monocytogenes* in just 24 h from both food and environmental surface samples.

Purpose: The two methods were individually validated using the Harmonized AOAC Official Method Pre-collaborative Study and AOAC Research Institute (AOAC RI) Performance Tested Method (PTM) requirements.

Methods: Method comparison studies for the Assurance GDS for *Listeria monocytogenes*.

Results: Results showed Assurance GDS for *Listeria monocytogenes* (GDSLm) and Assurance GDS for *Listeria* spp. (GDSLs) are equivalent to the reference culture methods for the detection of *L. monocytogenes Listeria* spp. (for GDSLs) in selected foods and environmental surfaces. In these studies, we observed 100% inclusivity among the 50 *L. monocytogenes* serovars (or *Listeria* spp.) and 100% exclusivity for the 31 non-*L. monocytogenes* (or non-*Listeria* spp.) organisms analyzed.

Significance: Assurance GDS for *Listeria monocytogenes* and Assurance GDS for *Listeria* spp. are two accurate and sensitive rapid methods for the detection of *Listeria* in selected foods and environmental surfaces.

T4-02 Using Stakeholder Input to Define Knowledge Areas Needed for a Curriculum in Food Protection and Food Defense

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Introduction: Despite the importance of equipping the next generation of professionals to protect our nation's food supply, educators face an absence of comprehensive data on which to base food protection and defense curriculum development efforts.

Purpose: This project identified the desired skills, knowledge, behavior, and attitudes upon which to develop a graduate level and professional development curriculum for food protection and defense.

Methods: A three-day Developing A CurriculUM (DACUM) workshop was conducted, during which an expert panel representing key stakeholders with expertise in food science; food safety; food manufacturing, distribution and retail industries; regulatory issues; epidemiology; law enforcement; homeland security; and public health were assembled to identify knowledge domains, learning objectives, and core educational competencies required for food defense professionals. The knowledge domains identified during the workshop were then compiled into an electronic follow-up survey and distributed to a larger group of stakeholders and professionals (n = 297) who were asked to rate each of the more than 100 items according to importance and frequency of use.

Results: The panelists participating in the DACUM workshop identified key areas of competence that related to preventing, detecting, diagnosing, responding to, and recovering from a food system incident; the workshop participants also identified communication and research as important elements. Data from the follow-up survey corroborated the relevance of the competencies identified during the DACUM workshop, as mean importance ratings for the various knowledge areas ranged from 3.4 to 4.6 on a scale of 1 (not at all important) to 5 (extremely important). Examples of competencies receiving some of the highest mean importance ratings included those associated with assessing vulnerabilities within a food system, developing and implementing food defense plans, implementing security and defense measures, and responding to food system incidents.

Significance: Results have provided a comprehensive set of knowledge domains and critical core educational competencies related to food protection and defense that can serve as a foundation for educational curricula for both graduate education programs and professional development and training programs.

T4-03 Self-reported Adoption of Food Safety Habits after Completing a Certified Food Manager Course: Does Education, Years of Foodservice Experience or Job Responsibility Make a Difference?

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Introduction: Research suggests that individuals who complete a Certified Food Manager (CFM) course improve their food safety habits. Whether this improvement varies by education, years of work experience or current job responsibility is unknown.

Purpose: This study examined the extent to which changes in food safety behaviors by CFM program participants varied by education, years of food service experience or current job responsibility.

Methods: Individuals completing a CFM program (n = 829) responded to a retrospective post survey that assessed how often they practiced selected food safety behaviors. Years of work experience, level of education completed and current job responsibility also were examined. Behavior change was examined, using paired samples *t*-tests and Tukey's HSD post hoc analysis.

Results: Most participants were female (75%), white (62%), and between 40 and 59 years of age (58%). Ten percent had not finished high school, but nearly 16% had a college degree. On average, participants had 11 years food service experience. More than one-third (36%) of participants identified themselves as a manager or supervisor while 25% reported being the owner of their establishment. Less than 20% identified themselves as a food preparer. Data analysis revealed significant ($P < .05$) improvements in all behaviors examined. When improvement in behavior was examined by level of education, job responsibility, and years of experience, significant differences were identified. For example, the amount of improvement in storing raw foods below those already prepared, using the two-stage cooling method, and hand washing varied significantly by years of experience.

Significance: For these individuals, the CFM program was effective in improving selected food safety behaviors. For some behaviors, the extent to which improvement was made varied by job responsibility, work experience, and/or level of education. Because the CFM is often the food establishment's "expert" on food safety and may be responsible for teaching food safety to employees, it is critical that establishments give careful consideration when selecting employees for the CFM role.

T4-04 The Economic Cost of Health Losses from Foodborne Illness

DSC

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Introduction: The economic costs of foodborne illness are not easily measured. This is true because the vast majority of cases identified by Mead et al. (1999) require no formal medical care. Nevertheless, costs to individuals in terms of lost productivity, limited mobility, and pain and suffering are legitimate costs that should be factored into policy decisions related to foodborne illness.

Purpose: The purpose of this study was to evaluate the economic cost of foodborne illness for the United States as a whole and for each of the pathogens identified by Mead et. al. in particular. The goal is to derive estimates that can be used to inform policy choices.

Methods: The enhanced food safety cost of illness model (Scharff et. al. 2009) is used to derive national estimates for the cost of illness in the United States. An innovation of this model was to include quality of life losses in addition to the medical and productivity losses that have been used in the past. This is an important enhancement, given that the vast majority of illnesses reported by Mead involve no use of formal medical care. A significant improvement to the basic model developed by Scharff et al. involves the use of weighted cost estimates which incorporate geographic differences into both the cost of illness and the incidence of illness to derive more accurate estimates. @Risk 4.5 is used to perform a sensitivity analysis that characterizes uncertainty and provides alternative specifications of the model based on differing assumptions.

Results: Under the most likely scenario, the annual expected cost of foodborne illness in the United States is approximately $112 billion ($45 to $180 billion in a 95% C.I.). This estimate is 100% greater than estimates based on older models that neglect to place a value on quality of life losses. The future inclusion of consequences from chronic sequelae is expected to significantly increase the magnitude of this figure.

Significance: The current economic crisis has led Federal, state, and local legislatures to consider cuts in all types of programs, including those that provide public health benefits. Food safety programs are not immune to this threat. For this reason, economic justification of food safety research and interventions is increasingly important.

T4-05 Food Safety Training Need Assessment for Independent Ethnic Restaurants: Review of Health Inspection Data in Kansas

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Introduction: Dining in restaurants is an important part of life in the US, as is evident in the more than 70 billion meal/snack occasions served annually. The majority (52%) of foodborne disease outbreaks have been attributed to restaurants or delicatessens. Further, the inspection scores of independent ethnic restaurants are routinely lower than non-ethnic or chain restaurants, indicating that additional food safety training for ethnic restaurants is needed.

Purpose: The purpose of this paper was to identify specific food safety training needs for independent ethnic restaurants, based on detailed online health inspection data.

Methods: Five hundred randomly selected independent restaurant inspection reports (250 ethnic/250 non-ethnic) in 14 Kansas counties with large numbers of Hispanic and Asian populations were reviewed. Frequencies of 275 Food Code violations were recorded and grouped into categories for further analyses. Independent sample t-tests were used to compare numbers of critical and non-critical violations, inspections within 12 months, and violations within each category.

Results: The five most prevalent violation categories regardless of restaurant type were time and temperature control of potentially hazardous foods (PHF), physical facility maintenance, protection from contamination, control of hands, and proper use of utensils. Ethnic restaurants had more critical (4.5 ± 3.0) and non-critical violations (2.8 ± 2.9) and more frequent inspections (2.3 ± 1.6) than non-ethnic restaurants (2.9 ± 2.8, 1.7 ± 1.9, & 1.8 ± 1.1, respectively, $P < 0.001$). Significantly ($P < 0.05$) more violations were reported in ethnic restaurants for the following categories: time and temperature control of PHFs, physical facility maintenance, protection from contamination, control of hands, proper use of utensils, demonstrated knowledge, and food temperature control for non-PHF.

Significance: The data suggest that ethnic restaurant personnel have increased needs for food safety training, more specifically for critical behaviors such as time and temperature control and hand washing. Food safety training may also need to be provided in their native language to increase understanding and effectiveness.

T4-06 DSC Application of Ionic Liquids for Separation and Concentration of Foodborne Pathogens from Food for Subsequent Molecular or Cultural Quantification Methods

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Introduction: The implementation of critical pathogen levels for foodstuffs will necessitate pathogen direct quantification as a standard procedure in food risk analysis. Until now, major challenges for biomolecular detection and quantification (such as real-time PCR) of foodborne pathogens have been heterogeneous food matrices and large sample quantities. Therefore, a major research topic is the development of sample treatment methods prior to subsequent molecular detection and quantification methods, which allow the separation of the target organisms from the sample matrix. Because of their unique physicochemical properties, ionic liquids offer a promising new approach contrary to classical microbiology.

Purpose: The purpose of this study was the development of a new sample treatment method for quantification of foodborne pathogens enabling subsequently both molecular and cultural methods for detection and characterization.

Methods: Several buffer compositions including ionic liquids were tested for their ability dissolving various edibles, without affecting the bacterial target cells. The toxicity of these buffers towards *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium were investigated. Quantification of both pathogens from artificially contaminated food samples was quadripartite carried out by either real-time PCR targeting the prfA-gene for *L. monocytogenes*, the fimA-gene for *S.* Typhimurium or selective plating methods, respectively.

Results: The application of 1-ethyl-3-methylimidazolium thiocyanate to the lysis buffer system enabled the quantifiable isolation of *S.* Typhimurium and *L. monocytogenes* from different artificial contaminated foodstuffs with decreasing inoculums ranging between 10^5 to 10^2 cells. Recovery for *S.* Typhimurium on selective agar plates varied between 45% (RSD

6%) out of 6.25 g egg and 36% (RSD 19%) out of 6.25 g ice-cream. *L. monocytogenes* was recovered with 67% (RSD 26%) from 12.5 ml milk and for both pathogens real-time PCR quantification resulted in higher (1.5 to 2 fold) bacterial equivalent counts than with CFU determination.

Significance: Application of ionic liquids permits the separation of foodborne bacterial pathogens from the food of interest for subsequent quantification with both real-time PCR and culture methods. Quantitative results can be obtained within one working day with the new buffer system.

T4-07 A Novel, Automated, Large Volume Re-circulating IMS Sample Processing Device for Rapid Isolation of Specific Pathogens from Pre-enriched Pooled Food Samples

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Introduction: Re-circulating IMS (RIMS) is well established as a robust and versatile approach to pathogen isolation from food and environmental samples, combining straightforward analysis of large, pooled pre-enriched samples, and removal of potential PCR inhibitory compounds with the ability to detect initially low levels of target pathogens. Utilizing the RIMS principle (the continuous flow and repeated passage of pre-enriched sample across a semi-permanent immobilized selective capture surface), PATHATRIX is widely employed in both routine food pathogen testing and outbreak investigations by regulatory agencies.

Purpose: Recirculating IMS has many desirable characteristics but involves a series of manual steps to achieve washing and recovery of magnetic particles. While this labor is minimal, a degree of user interaction is required. The availability of an automated device employing the RIMS principle will enhance the sample handling and processing involved in food pathogen testing. This study describes the development, evaluation and validation of a large volume IMS device that automates the capture, wash, elution and bead concentration steps. The aim of this study is to demonstrate comparative or better performance than the current manual RIMS method. The ability to link the automated RIMS system to real time PCR was also investigated.

Methods: A range of food samples, including several implicated in a significant number of food safety recalls and outbreaks during the past two decades (ground beef, peanut butter, chocolate, almonds and fresh produce) were inoculated (1 to 10 CFU/sample) with appropriate target pathogens (*E. coli* O157, *Salmonella* serovars, *Listeria* spp.). Samples were pre-enriched according to the standard RIMS protocols. Duplicate pooled samples of each food and pathogen combination were processed in parallel using automated RIMS alongside PATHATRIX . Both systems used immuno magnetic particles with the same magnetic properties, target specificity and inclusivity. Recovery of target pathogens after plating IMS beads onto selective agar plates was used as the detection methodology.

Results: The detection of a range of target pathogens from pooled food samples by selective agar plating and real-time PCR was achieved using a novel, automated RIMS device. A 100% correlation between the recovery and detection of target organisms by the automated and manual RIMS systems was observed.

Significance: The data presented in this study demonstrates that it is feasible to fully automate the RIMS procedure and employ standard PATHATRIX immuno-capture beads in this process with no loss of bead recovery or target pathogen capture functionality.

T4-08 Evaluation of PremiTest *Salmonella* for Rapid Serotyping of *Salmonella* Strains Isolated from Broiler Farms in Southern Brazil

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Introduction: Salmonella serotyping plays an important role in *Salmonella* reduction programs, as it facilitates identification of the source of contamination. The conventional method for serotyping (Kauffmann-White scheme) is complex, labor intensive and time consuming, and therefore is only remotely available for many routine laboratories. The PremiTest Salmonella is a novel and rapid alternative test for *Salmonella* serotyping; it recognizes 78 of the most frequently encountered serotypes, is intended for routine use and can be completed within one working day.

Purpose: The objective of this study was to evaluate the performance of the PremiTest Salmonella.

Methods: A total of 100 *Salmonella* strains isolated from Brazilian broiler farms, comprising 21 different serotypes, was serotyped, using both the conventional and the alternative method. The alternative method uses multiplex PCR and a micro array to determine a genetic profile of the isolate (genovar). The accompanying software translates the genovar into a regular serotype.

Results: The results indicate that 19 serotypes (n = 95) were recognized by the test; two serotypes (Corvalis and Rubislaw) were not present in the database, but were recognized with a distinct genovar code. From the 95 isolates, 85% was assigned to the right serotype (n = 81) and none of the strains was assigned to the wrong serotype. The remaining isolates (n = 14) yielded genovar scores that were not recognized by the software. Some of these scores were encountered for multiple strains and may be included in future versions of the software, thereby indicating the capabilities of the technique.

Significance: The study indicates that the PremiTest Salmonella is a rapid and promising alternative to the regular Kauffmann-White scheme. It is easy to use and makes serotyping available to routine laboratories. As such, it is a valuable tool in *Salmonella* reduction programs.

T4-09 Efficient Method for Developing Group-specific Primers for Feed Inspection, with Eight Examples of Species/Breed Group

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Introduction: To prevent a spread of BSE in cattle through feed, Japan introduced in October 2001 a feed ban prohibiting the use of animal proteins in feed. This feed ban was later altered to allow the use of proteins derived from fish, swine and poultry in feed for fish, swine and/or poultry. Thus, the feed ban is at a new stage, with some of the prohibited animal species being exempted for use as feed materials.

Purpose: To implement the feed regulation of prohibited species according to the BSE risks, primers screening the species, including all breeds, are essential. For developing group-specific primers more efficiently and swiftly, we revised our method.

Methods: Putting weight points on 3'-end of the primer sequence, difference between primer sequences out of target and non-target group sequences were scored. We tried some sets of the weight points, and found appropriate sets. The computerized calculation program, which we previously developed, was updated. It also solved a miscalculation problem caused by a gap in the multialignment. Ruminants group included 20 sequences of mitochondrial complete genome for deer, sheep, goats, pig, cetacean, TSE susceptible animal group, and krill/crub group included 9, 3, 2, 45, 9, 18 and 6 sequences, respectively.

Results: We developed a total of 12 primer sets for 6 species/breed groups (ruminants, deer, sheep, goats, pig, and cetacean), but failed to develop primers for the TSE group and krill/crub group. One of the primer sets for ruminants has been used for feed inspection in Japan. There has been no positive test result except for dairy products so far.

Significance: Although there are many types of deer and cetaceans, the primer sets commonly specific to these species have not been reported. Our method is useful in developing primers for screening feed or food for commingling of animal proteins.

T4-10 Comparison of Reverse-transcriptase Loop-mediated Isothermal Amplification (RT-LAMP) to RT-PCR and Cultural Methods for the Detection of *Salmonella* Typhimurium in Pork

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Introduction: Reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) is a novel molecular method that has advantages over PCR with nucleic acid amplification at one temperature within a few hours, eliminating the need for expensive equipment.

Purpose: Our objective was to compare the detection sensitivity of *Salmonella* Typhimurium from pork products by RT-LAMP, and by traditional assays.

Methods: Twenty-five gram pork-chop and sausage samples were inoculated with high (10^8 to 10^6 CFU) and low (10^3 to 10^1 CFU) inocula of *S.* Typhimurium and stomached in 225-ml tetrathionate broth. Serially diluted samples were plated on XLT4 agar, either immediately or after 10-h selective pre-enrichment at 37°C. Negative controls included un-inoculated pork samples and water; the positive control was *S.* Typhimurium. Each experiment was replicated twice. RNA was extracted from 1-ml samples, using the TRIzol® method. RT-LAMP assay using six described *Salmonella*-specific invA gene primers, Bst DNA polymerase and reverse-transcriptase was conducted at 62°C for 90 min in a water bath. Detection was by agarose gel electrophoresis and visual turbidity. Real-time RT-PCR analysis was carried out using a SYBR Green I kit with invA gene primers and an internal amplification control. Reaction conditions were 50°C/30 min and PCR at 95°C/30 s, 58°C/30 s, 72°C/30 s for 45 cycles in a BioRad iCycler, followed by melt temperature analysis.

Results: Rapid *Salmonella* detection up to 10^2 CFU/25 g for both pork samples by RT-LAMP assay was obtained after 10-h enrichment in comparison to 10^2 CFU/25 g pork and 10^4 CFU/25 g sausage by RT-PCR. Even without enrichment, *Salmonella* could be detected at 10^6 CFU/25 g for both samples by RT-LAMP, within one day, and at 10^6 and 10^7 CFU/25 g by RT-PCR, respectively. The traditional cultural assay, though highly sensitive, with positive results in samples at all tested *Salmonella* levels, takes several days.

Significance: This RT-LAMP assay shows promise for improved and rapid *Salmonella* detection from pork products within 24 h, with potential use in routine testing.

T5-01 Internalization of *Escherichia coli* O157:H7 in Spinach Cultivated in Soil and Hydroponic Media

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Introduction: Internalization of *E. coli* O157:H7 into spinach plants through root uptake is a potential route of contamination. Previous studies that have investigated uptake of *E. coli* O157:H7 into leafy greens have expressed green fluorescent protein (gfp) from a plasmid, possibly limiting detection of stressed cells in internal plant tissues.

Purpose: The objective of this study was to develop strains of *E. coli* that contain a chromosomal insert of the gfp gene, and assess the uptake of these strains into spinach plants.

Methods: A Tn7-based plasmid vector was used to insert gfpmut2 into the attTn7 site in the *E. coli* chromosome. Three gfp¬-labeled *E. coli* inocula, O157:H7 strains 4407 and 5279 (Inoculum 1), O157:H7 strain 86-24 h11 (Inoculum 2), and HS (Inoculum 3), were cultivated in fecal slurries and applied at ca. 3 log CFU/g or 7 log CFU/g to pasteurized soils or to hydroponic media in which baby spinach was planted.

Results: No *E. coli* inocula were recovered by spiral plating from surface-sanitized internal tissues of spinach plants on d 0, 7, 14, 21, and 28 (< 1.7 CFU/shoot or root). Inoculum 1 survived at significantly ($P < 0.05$) higher populations than Inoculum 3 after 14, 21 and 28 days in soil. Inoculum 2 applied to hydroponic medium at ca. 7 log CFU/ml were recovered from the shoots of spinach plants after 14 (3.73 log CFU/shoot) and 21 days (4.35 log CFU/shoot). Fluorescent *E. coli* cells were primarily visualized in root tissues by microscopy in plants grown in soils, but were not recovered by spiral plating.

Significance: The stress encountered in the rhizosphere and intact plant tissue prevents the uptake of *E. coli* O157:H7 from soils to spinach plant shoot tissue. No differences in the ability of O157:H7 and commensal *E. coli* to internalize to spinach plants were observed.

T5-02 Attachment of *Salmonella* spp. to Intact and Cut Produce Surfaces

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Introduction: Salmonella outbreaks have been associated with the consumption of fresh produce. The produce may be contaminated with *Salmonella* at any point throughout the food continuum. To develop effective strategies to minimize the risk of foodborne disease caused by this organism, it is essential to examine initial stages of bacterial attachment to various plant tissues.

Purpose: The purpose of this study was to evaluate the attachment of *Salmonella* spp. to different produce surfaces.

Methods: Three types of fresh produce (lettuce: romaine and iceburg, cabbage) were used in the study. Two configurations of coupons: 2-cm disk shaped produce coupons (intact surface) and 2 × 0.5-cm strips cut from mid-vein of leaf at the thickest point near the base of produce (cut surface) were used. Coupons/strips (n = 360) were submerged into four individual *Salmonella* spp. and stored at 10°C for up to 24 h. Samples were removed periodically and analyzed for loosely attached cells (those removed when samples were vortexed for 20 s in PBS/Tween20) and strongly attached cells (those removed when samples were homogenized for 20 s using high speed homogenizer).

Results: Attachment to produce surfaces among *Salmonella* spp. was variable. Attachment of *S.* Tennessee to produce surfaces was significantly greater than the attachment of *S.* Negev. *Salmonella* attachment to cabbage was significantly lower than the attachment to romaine or iceburg lettuce. Most *Salmonella* were attached to cut surface at levels 0.4-1 log CFU/ml above numbers on intact surface. However, the difference between bacterial attachment to intact and cut surfaces was not significant for lettuce. Populations of loosely attached *Salmonella* on cabbage and iceburg lettuce surfaces were significantly greater than populations of strongly attached bacterial populations on these produce. Most bacterial attachment occurred during the first hour of exposure to produce surface.

Significance: The study shows the preferential attachment of *Salmonella* to produce surfaces. Further, *Salmonella* binds rapidly to plant tissues irrespective of the type of produce. Results may aid in understanding the contamination process and in developing effective mitigation strategies.

T5-03 Effect of Fresh Produce Crop Residue on the Survival of *Escherichia coli* O157:H7 in Soil

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Introduction: Significant amounts of un-harvested crop residues are incorporated into the soil at the end of the growing seasons on leafy green produce fields. The extent to which these plant remnants contribute to persistence of soil and produce contamination by bacterial pathogens during the subsequent growing season is unclear.

Purpose: The purpose was to evaluate the effects of decomposing plant residues on the survival and proliferation of *E. coli* O157:H7 in soil.

Methods: Spinach leaves and soil were inoculated with several *E. coli* O157:H7 strains and buried in soil with leaves of cabbage, tubers of forage radish, or spinach. Survival and proliferation of inoculated pathogenic strains was measured for 35 days during biomass decomposition using MPN procedures.

Results: No significant proliferation of *E. coli* O157:H7 strains occurred during the 5 week biomass decomposition period. However, *E. coli* O157H7 survival was significantly affected by type of decaying biomass. When *E. coli* O157:H7-inoculated spinach leaves were exposed to cabbage biomass, the pathogen population declined by 5.0 log after 35 days of decomposition in soil, compared with 3.4, 3.0, and 3.3 log when exposed to biomasses of spinach, radish tubers, or soil without added biomass, respectively. Survival of *E. coli* O157:H7 inoculated from bovine fecal solids was not significantly affected when different types of crop biomass were tested.

Significance: Cabbage and other brassicaceous plants have robust glucosinolate-myrosinase systems. Several glucosinalate hydrolysis products released during decomposition may reduce survival of bacterial pathogens under certain conditions (soil biofumigation). Data from this study suggests that biofumigation in conjunction with Brassica plant rotation could be an effective means of mitigating field contamination by bacterial pathogens in leafy green production systems.

T5-04 DSC Transfer of *Escherichia coli* O157:H7 from Equipment Surfaces to Iceberg and Romaine Lettuce during Simulated Commercial Processing

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Introduction: Cross-contamination/multi-directional transfer of *Escherichia coli* O157:H7 during commercial shredding, conveying, fluming and drying of fresh-cut leafy greens has become a major public health concern, particularly because post-processing kill steps are currently not implemented in the industry.

Purpose: The goal of this study was to quantify *E. coli* O157:H7 transfer from product-inoculated equipment surfaces to uninoculated lettuce during commercial processing.

Methods: Three to five batches of locally purchased retail iceberg and romaine lettuce (136 kg/batch) were cored, after which 22.7 kg of product was dip-inoculated to contain ~10^6, 10^4 or 10^2 CFU/g of a 4-strain avirulent, gfp-labeled, ampicillin-resistant *E. coli* O157:H7 cocktail. Thereafter, 22.7 kg of each uninoculated product was processed, using a

Urschel TransSlicer shredder, step conveyor, 3.3-m flume tank, shaker table and centrifugal dryer (22.7 kg capacity), followed by 22.7 kg of inoculated product during which time ~5 kg of each product was collected in a time series of baskets. After draining the flume tank, 11 known product contact areas on the equipment (100 cm^2) were sampled using Kimwipes®. After refilling the flume tank, 90.8 kg of uninoculated product was similarly processed and collected in ~5 kg aliquots. After processing, 42 equipment surface samples along with 46 iceberg and 36 romaine lettuce samples (25 g each) from the collection baskets were diluted in phosphate buffer and examined for gfp-labeled *E. coli* O157:H7 by direct plating or membrane filtration using trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin.

Results: Initially, the greatest *E. coli* O157:H7 transfer was seen from inoculated lettuce to the shredder and conveyer belt, with all equipment surface populations decreasing 90 to 99% after processing 90.8 kg of uncontaminated product. When product containing 10^6 and 10^4 *E. coli* O157:H7 CFU/g was processed followed by uninoculated product, *E. coli* O157:H7 was quantifiable in all subsequent iceberg and romaine lettuce samples (90.8 kg), respectively. At the 10^2 CFU/g inoculation level, *E. coli* O157:H7 was consistently detected after processing 21.2 and 68.0 kg of previously uninoculated iceberg and romaine lettuce at average levels of 3 and 2 CFU/100 g and transferred out to 75.6 and 86.9 kg at 4 CFU/100 g, respectively.

Significance: These findings, which clearly demonstrate the ability of *E. coli* O157:H7 to persist on equipment and subsequently contaminate potentially large quantities of previously uncontaminated fresh-cut leafy greens during processing, are critical to the development of science-based transfer models for risk analysis.

T5-05 Assessment of Preharvest Internalization of Bacteria into Melons from Irrigation Water

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Introduction: Melons harbor endophytes, including enteric bacteria, within the vine and these may regularly enter fruit at very advanced stages of ripening. The risk of systemic transfer of human pathogens has not been determined.

Purpose: Evaluate preharvest potential for dose-dependent uptake of avirulent *E. coli* O157:H7, *Salmonella* and a surrogate *Citrobacter* in controlled environments.

Methods: Challenge bacteria were inoculated at four concentrations to the soil or rhizosphere. Cantaloupe ("Oro Rico" F1) and Honeydew ("Summer Dew" HMX 4593) seeds were planted in horticultural mix and inoculated at stages from germination to flowering. Inoculation was by sub-irrigation. "Contamination" levels were uninoculated, or log 4, 6 or 7 CFU/ml of water applied. Following surface sterilization, recovery was by selective enrichment and plating.

Results: Recovery of internalized *E. coli* O157:H7 after one day was detectable within 0, 10 and 30 percent of cantaloupe and 0, 10 and 50% of honeydew at Log 4, 6 and 7, respectively. The surrogate *Citrobacter youngae* was detectable among 20, 40 and 20 percent of cantaloupe and 10, 0 and 20% of honeydew at log 4, 6 and 7, respectively. After one week, recovery was 0, 40 and 0 percent of cantaloupe and 10, 0 and 10 percent of honeydew at log 4, 6 and 7, respectively. At two weeks, recovery was limited to 10 percent of cantaloupe from dose Log 7. With *Salmonella,* recovery was obtained in 1 of 30 honeydew at this highest dose. Systemic transport was limited to the first internode. Two weeks following contamination, with high levels of viable bacteria in the rhizosphere, internalization was observed.

Significance: The outcomes of these model system experiments indicate that, upon a contamination event of a high dose of bacteria into the soil via irrigation water, there exists the potential for immediate, but apparently transient, transfer of enteric pathogens into melon vines.

T5-06 Influence of Irrigation Methods on Coliform Internalization in Blueberries

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Introduction: Higher than expected coliform results were observed during a 2007 crop year efficacy study of various chemical sanitizer washes for frozen blueberries. Initial analysis identified irrigation water as the source of coliform contamination but traditional countermeasures were unsuccessful at reducing coliform presence.

Purpose: Twenty US blueberry fields were surveyed in 2008 to determine relevance of irrigation practices to the incidence and location of coliform bacteria of fresh blueberries. Blueberries were irrigated with over-head sprinklers from four water sources: private well, pressurized irrigation district, surface water, and calcium hypochlorite treated surface water.

Methods: Fruit samples (n52) were collected from lugs upon delivery. Whole sound fruit samples (10 g diluted with 90 ml buffered solution) were prepared as follows (1) External rinse with buffered solution was done (2) fruit was digested and added to buffered solution, and (3) fruit (after plating from (1) above) are drained and exposed to UV light for 120 s, digested and added to buffered solution. Samples were plated on *E. coli* / Coliform Petrifilm.

Results: Coliforms were found in 15% of fruit subject to external rinse (1) and 35% of whole fruit representing internal and external surfaces (2) where 90% of those samples with coliforms in preparation (1) also had coliforms in preparation (2). Coliforms were found in 25% of UV surface sterilized fruit (3); notably, when data points are linked to only those samples where coliforms were found in either (1) and (2), coliforms were found in 46% of samples. Coliforms were not found on fruit from growers with private wells or treated surface water irrigation systems.

Significance: Findings suggest coliform bacteria can migrate from irrigation water into internal fruit tissue where it is protected from traditional sanitizing wash water applications. Therefore, handlers of fresh and frozen blueberries should employ best irrigation practices at the farm level rather than relying on sanitizing wash water.

T5-07 Biocontrol of *Salmonella* in Developing Tomato Fruit with a Combination of Lytic Bacteriophages and Antagonistic Bacteria

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Introduction: It has been demonstrated that *Salmonella* introduced onto the blossom of tomato plants can contaminate the internal and external parts of the subsequent fruit. Once internalized, the *Salmonella* cannot be removed by washing and hence represents a significant food safety risk. The use of chemical sanitizers to decontaminate the blossoms of tomato plants is impractical, ineffective and non-specific. Therefore, alternative approaches are required to control *Salmonella* in the field or greenhouse to enhance the microbiological safety of tomatoes.

Purpose: The study evaluated the efficacy of a biocontrol preparation composed of antagonistic bacteria and lytic bacterio-phages, which were applied to the blossoms of tomato plants to prevent contamination of the subsequent fruit.

Methods: The biocontrol preparation consisted of a *Enterobacter asburiae* strain isolated from mung bean sprouts and a cocktail of five *Salmonella* infecting bacteriophage. In vitro trials were performed by co-inoculating the phage and *E. asburiae* with a 5 strain cocktail of *Salmonella* in Tryptic Soy Broth (TSB) and monitoring levels of the different microbes over time. Further experiments co-inoculated biocontrol preparation with *Salmonella* Javiana onto the blossoms of tomato plants. The tomatoes were allowed to develop over a 7-week period and subsequently screened for *Salmonella*, *E. asburiae* and phages.

Results: When applied individually, *E. asburiae* and bacteriophage could reduce the growth of *Salmonella* in TSB by 3 log CFU/ml or 2 log CFU/ml respectively. However, *E. asburiae* and bacteriophage applied in combination reduced *Salmonella* populations to < 0.1 log CFU/ml. Tomato plants inoculated with *Salmonella* produced 92% (22/24 batches positive) surface and 43% (31/72 fruit positive) internally contaminated fruit. In the presence of the biocontrol preparation, the prevalence of *Salmonella* associated with tomatoes was significantly ($P < 0.05$) reduced. No *Salmonella* were recovered on the surface of tomatoes, with only 2% (1/56 fruit positive) being contaminated internally.

Significance: The developed biocontrol preparation offers a potential route for controlling *Salmonella* in the field environment, thereby enhancing the microbiological safety of tomatoes.

T5-08 Efficacy of Gaseous Chlorine Dioxide as a Postharvest Disinfectant for Stone Fruit

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Introduction: Non-aqueous surface disinfectants to control postharvest decay and as a proactive risk reduction practice are of interest to handlers and distributors of stone fruit, including peaches, plums, and nectarines.

Purpose: Develop preliminary efficacy data towards the establishment of a Ct-value for gaseous chlorine dioxide on stone fruit to maximize lethality to *E. coli* O157:H7 and *Salmonella* spp. without a reduction in fruit quality.

Methods: Freshly harvested fruit were spot-inoculated with 10 µl of a 2 to 3 strain cocktail of *E. coli* O157:H7 or *Salmonella* at log 8.0 CFU/ml. Fruit surfaces were inoculated directly or after mild abrasion to simulate micro-wounding associated with harvest operations. The inoculated fruits were air-dried for 18 h at 20°C. Sachets containing chlorine dioxide formulations, provided by ICA TriNova, LLC, were placed in chambers with water for precursor activation. Fruit was exposed at 0°C or 2.5°C for up to 7 days. The inoculated area was excised, placed in neutralizing buffer, and plated on appropriate selective media. Recovery was conducted with or without a resuscitation step, using a polycarbonate membrane transfer protocol. Viability assessments were also conducted, using Assurance GDS kits and protocols.

Results: On nontreated fruit the mean log reduction after the post-drying viability loss was 0.6 or less under storage conditions. The cumulative concentration of ClO_2, determined from a release-curve graph, was approximately 9.2 mg/L, 18.4 mg/L, and 27.5 mg/L, respectively over 7 days at 2.5°C. Fruit exposure at 0°C was 0.5 mg/L, 2.1 mg/L, and 3.7 mg/L of ClO_2. Log reduction on treated fruit ranged from 1.8 to greater than 5.0, depending on the fruit and specific test parameters. Log reduction compared to nontreated fruit was greatest for plums.

Significance: Preliminary assessments indicate that gaseous chlorine dioxide has potential as an efficacious postharvest disinfection treatment to minimize risk of foodborne pathogens on stone fruit without inducing visual defects or quality loss.

T5-09 Hot Water Surface Pasteurization vs. Chlorine Wash for Reducing Populations of *Salmonella* Poona on Artificially Inoculated Tomatoes

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Introduction: Numerous outbreaks of salmonellosis have been associated with the consumption of fresh 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: Fresh green tomatoes, dip inoculated with *Salmonella* Poona RM 2350 or to a final cell concentration of ca. 5 log CFU/g were stored at 4 and 20°C for up to 24 h prior to processing. Six inoculated tomatoes were treated for 3.5 min in 70°C water or 20 ppm chlorine solution, and individual tomatoes was blended in peptone water (1:1) for 1 min. Enumeration of non-injured cells was done by plating on the selective media XLT-4 incubated at 35°C overnight. Injured cells were recovered by plating on non-selective media TSA and incubating at 35°C for 2 h after which plates were overlaid with XLT-4 and were incubated at 35°C overnight.

Results: Reductions in *S.* Poona populations following treatments in water at 70°C and 20 ppm chlorine solution were 6 and 1 log CFU/g, respectively. Hot water treated tomatoes that were stored at 20°C for 5 days showed normal maturation

process with no obvious visual injury. Storage of untreated inoculated tomatoes at 13, 20, and 30°C for up to 6 days post inoculation caused a significant ($P < 0.05$) increase in *S.* Poona populations (up to 3 log CFU/g) as compared to storage at 4°C.

Significance: These results indicate that surface pasteurization will enhance the microbiological safety of tomatoes with no adverse effect on the maturation process and will extend the shelf life of this commodity as well. Storage data indicated that tomatoes should be refrigerated as soon as possible following harvesting to suppress the growth of any possible contaminant.

T5-10 Sanitizer Solutions Containing Detergents for Inactivation of *Escherichia coli* O157:H7 on Romaine Lettuce

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Introduction: Numerous *Escherichia coli* O157:H7 outbreaks have been linked to consumption of fresh lettuce. The development of effective and easily implemented wash treatment could reduce such incidents.

Purpose: The purpose of this study was to evaluate the addition of food-grade detergents to sanitizer solutions for inactivation of *E. coli* O157:H7 on romaine lettuce.

Methods: Freshly-cut leaves of romaine lettuce were dip-inoculated to achieve a final cell concentration of 7.8 ± 0.2 log CFU/g, air-dried for 2 h, and stored overnight at 4°C. Leaves were then washed for 2 min in an experimental short chain fatty acid formulation (2CL) or in one of the following solutions with or without 0.2% dodecylbenzenesulfonic acid or 0.2% sodium 2-ethyl hexyl sulfate: 1) deionized water; 2) 100 ppm chlorine dioxide; 3) 100 ppm chlorine; 4) 200 ppm chlorine. Following wash treatment, samples were blended in neutralizing buffer (1:3) and surface plated on the selective medium CT-SMAC.

Results: The efficacies of wash treatments, with or without the detergents, in inactivating *E. coli* O157:H7 cells on lettuce leaves were not significantly different. The most effective wash solution was 2CL, which was capable of reducing *E. coli* O157:H7 populations by more than 5 log CFU/g (none detected by direct plating). The rest of the wash treatments resulted in a population reduction of less than 1 log CFU/g.

Significance: The effectiveness of 2CL surpasses that of other sanitizer treatments tested in this study and requires further research to optimize treatments to preserve lettuce quality. Conventional detergents did not enhance the efficacy of any of the wash treatments tested during this study. The addition of such detergents to a conventional commercial wash treatment might not be advantageous.

T5-11 Pinpointing Sources of Contamination on Leafy Green and Fresh Market Tomato Farms

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Introduction: Leafy greens and fresh market tomatoes have been the source of high profile outbreaks. The causes of these outbreaks are often traced back to the farm; however, information is limited as to the key sources of on-farm contamination.

Purpose: To identify key areas of concern on leafy green and fresh market tomato farms, from growth to packing, such as in the field, at harvest, washing, hydro-cooling and packing; and to link environmental sources of contamination to the contamination found on the produce.

Methods: Four farms were involved in the study. Samples of produce in the field, at harvest, after hydrocooling, washing and packing from each farm, were obtained along with corresponding environmental samples and tested for generic *E. coli*. Environmental samples consisted of irrigation water, soil, hand swabs, post harvest water, harvest bin swabs, knife swabs, conveyor belt swabs and ice samples. Positive samples for *E. coli* were genotyped using Amplified Fragment Length Polymorphism (AFLP), and similarity between AFLP fingerprints among sample types were calculated using the band-based "Dice coefficient", enabling identification of contamination sources.

Results: For all four farms, a total of 2,098 samples were analyzed for generic *E. coli*. *E. coli* isolates were very diverse on each farm, with 309 (lettuce farm), 689 (tomato farm), 1,002 (parsley farm), and 1,968 (spinach farm) different isolates identified. For each farm, the points of harvest and beyond were the most significant areas of concern. Genotyping results demonstrated that pre and post harvest water, along with conveyor belts and hands, are the main sources of contamination.

Significance: These results provide data to policy makers, risk assessors, risk managers and farmers in terms of the areas on leafy green and fresh market tomato farms that are likely to affect food safety.

T5-12 Attachment of Norovirus in Manure and Biosolids to Lettuce
DSC

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Introduction: Norovirus is a major foodborne pathogen, with outbreaks widely associated with fresh produce. The attachment of norovirus to plants in fields may be a cause of contamination of fresh produce. Knowledge of how norovirus in biosolids/manures attaches to lettuce and the mechanisms of internalization into plant tissue is needed to protect public health.

Purpose: To study the attachment behaviors of norovirus recovered from biosolids and manure, specifically addressing virus attachment and internalization associated with lettuce leaves.

Methods: Murine norovirus-1 (MNV) was labeled with SYBR gold, and lettuce pieces were dipped into fluorescent MNV, agitated for 5 min, incubated at 4°C for 1 h, and analyzed with use of a confocal microscope. MNV was incubated in biosolids, solid swine manure (SM) and liquid dairy manure (DM) at 4°C for 10, 20 and 30 days. Manure samples were diluted 100-fold in phosphate buffer, and lettuce pieces were dipped and incubated at 4°C up to 24 h. MNV was recovered from the lettuce with HBSS buffer and analyzed by use of the plaque assay.

Results: With use of confocal microscopy, MNV was observed both on the surface and attaching to the cut edge of lettuce pieces. The guard cell was important in internalization, as MNV was observed inside it and in the tissue around it. The attachment percentage for pure MNV was ~0.02%; however, it increased 50-fold for MNV recovered from biosolids and 5-fold for SM, although it had no significant effect for MNV in DM ($P < 0.001$). After attachment, MNV was stable on lettuce and there was no significant reduction after a 24-h incubation for all samples ($P < 0.05$). The incubation time of MNV in manure and biosolids did not affect attachment, as no significant difference was observed between day zero and day 30 manure samples ($P < 0.05$).

Significance: This is the first study to observe virus recovered from manure/biosolids and subsequent attachment and internalization to lettuce by use of confocal microscopy.

T6-01 Food Commodities Associated with *Salmonella* Enteritidis Outbreaks 1998–2007

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Introduction: Salmonella serotype Enteritidis has been implicated as a major contributor to foodborne illness. An updated analysis evaluating the roles that various food commodities play in outbreak causation will provide useful insight for the development of control strategies.

Purpose: Provide updated data on the roles of food commodities as vehicles of transmission for *Salmonella* serotype Enteritidis.

Methods: We examined data on confirmed *Salmonella* serotype Enteritidis foodborne outbreaks with two or more illnesses investigated by state and local health agencies and reported to CDC during 1998-2007. Food items implicated in outbreaks were classified into one of seventeen food commodity categories (fish, crustaceans, mollusks, dairy, eggs, beef, game, pork, poultry, grains-breads, oils-sugars, fruits-nuts, fungi, leafy vegetables, root vegetables, sprouts, and vine-stalk vegetables).

Results: A total of 391 single-etiology outbreaks due to *Salmonella* serotype Enteritidis were reported to the CDC between 1998 and 2007, resulting in 10,019 illnesses, 932 hospitalizations, and 14 deaths. Of these, 132 (34%) outbreaks implicated food items that could be classified into a single commodity, with the top three commodities being eggs (64% outbreaks, 62% illness), poultry (17% of outbreaks, 20% illness), and fruits-nuts (5% outbreaks, 6% illness), none of which showed statistically significant changes in frequency over time. The median size of outbreaks during this time period was greatest for fruits-nuts (28 persons), followed by poultry (15 persons) and eggs (12 persons), and did not show statistically significant changes over time.

Significance: Salmonella serotype Enteritidis infections due to egg, poultry, and fruit-nut commodities comprised 86% of outbreaks due to a single commodity. Outbreaks of *Salmonella* serotype Enteritidis associated with eggs continue to occur, and eggs are the most frequently implicated commodity, indicating that control efforts are needed along the farm-to-fork continuum.

T6-02 Withdrawn

T6-03 Exploring Historical Canadian Foodborne Outbreak Datasets for Human Illness Attribution

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Introduction: Human illness attribution has been recently recognized as an important tool for developing a risk-based approach to manage the food supply. Analyzing outbreak datasets has been suggested and used in some countries for that purpose.

Purpose: This study explored three Canadian comprehensive foodborne outbreak datasets covering 30 years for their usefulness in estimating food attribution for gastrointestinal illness and provides Canadian food attribution estimates with a historical perspective.

Methods: Information on the microbiological causative agent and the food vehicles recorded for each outbreak was standardized between the three datasets. The agent-food vehicle combinations were described and analyzed for changes over time by using multiple correspondence analyses.

Results: Overall, 6,908 foodborne outbreaks were available over three decades (1976 to 2005) but the agent and the food vehicle were precisely identified in only 2,107 of them. Differences existed between the datasets in the distribution of the cause, the vehicle, the location or the size of the outbreaks. Multiple correspondence analyses showed association between *Clostridium botulinum* and wild meat and between *C. botulinum* and seafood. It also highlighted changes in food attribution over time. It generated the most up-to-date food attribution values for salmonellosis (29% produce, 15% poultry, 15% meat other than poultry, pork and beef), campylobacteriosis (56% poultry, 22% dairy products other than fluid milk), and *Escherichia coli* infection (37% beef, 23% cooked multi-ingredient dishes, 11% meat other than beef, poultry, pork) in Canada. Because of the inherent limitations of this approach, only the main findings should be considered for policy-making. The use of other human illness attribution approaches will provide further clarification.

Significance: This work is the first attempt to determine human illness food attribution from Canadian data. Multiple corres-pondence analyses was used to help clarify and visually highlight the food-pathogen associations contained in the causative agent and food vehicle cross-tabulation, while showing any change over time.

T6-04 Enumeration of *Campylobacter* on Chickens at Processing and Retail: An Explanation of Regional Differences in Incidence of *Campylobacter* Observed in Foodnet Sites

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Introduction: Over a million persons are infected with *Campylobacter* in the United States each year. Consumption of poultry is an important source of infection. The Foodborne Diseases Active Surveillance Network (FoodNet) measures the incidence of human *Campylobacter* infections in participating states. There are substantial variations in incidence between states, with higher incidence seen in the west.

Purpose: To explore this variation, we compared the *Campylobacter* prevalence on chickens purchased at retail in the FoodNet sites to chickens collected at processing plants during the United States Dept. of Agriculture/Food Safety Inspection Service (FSIS) Young Chicken Baseline Study.

Methods: During June to August 2008, fresh skin-on whole chickens were purchased at retail in nine FoodNet states. The plant code and sell-by date were recorded. The FSIS Young Chicken Baseline Study was conducted from June 2007 to July 2008. Chickens were rinsed in buffered peptone water and rinsates were enriched in Bolton broth and streaked onto Campy Cefex Agar. *Campylobacter* colonies were enumerated using direct plating onto Campy Cefex Agar.

Results: For the FoodNet study, 270 chickens were purchased; 241 were analyzed. Of these, 143 (59%) yielded *Campylobacter* (range 1 to 310 colony forming units[CFU]/mL). The percentage of chickens which yielded *Campylobacter* varied by site from 75% in Tennessee and 68% in California to 44% in New York. In the FSIS Young Chicken Baseline Study, 65 (41%) of 159 chickens collected at processing plants from which the retail chickens tested in the FoodNet study originated yielded *Campylobacter* (range 1 to 3400 CFU/mL). Ninety percent of chickens from corresponding production plants in California yielded *Campylobacter*, compared with only 22% of chickens from plants in Connecticut.

Significance: Regional differences in the incidence of *Campylobacter* infections may be partially explained by variations in Campylobacter prevalence on retail chickens. Further studies are needed to confirm these findings and to better understand contributing factors.

T6-05 Reduction in Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) *Salmonella* Positives at United States Food Safety and Inspection Service (FSIS)-regulated Broiler and Turkey Establishments

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Introduction: The increase in broiler samples positive for *Salmonella* collected under PR/HACCP testing (from 11.5% in 2002 to 16.3% in 2005) prompted FSIS to introduce a risk-based *Salmonella* sample scheduling initiative in 2006. This initiative included the introduction of a risk-based algorithm to schedule *Salmonella* sampling, the categorization of establishments depending on the level of process control, as determined by *Salmonella* set results, and the introduction of PR/HACCP testing for turkey carcasses.

Purpose: The numbers of *Salmonella* positives and the proportion of human health-hazard serotypes (hereafter "HHS") as identified by CDC were compared for the first two years of risk-based sampling July 2006 to June 2007 (year 1-risk based:Y1RB) and July 2007 to June 2008 (year 2-risk based:Y2RB)) and the preceding year (non-risk based sampling: NRB) July 2005 to June 2006. Furthermore, the FSIS goal of having 90% of regulated establishments in category 1, i.e., demonstrating consistent process control by 2010, was evaluated.

Methods: SAS software was used to extract, clean and analyze data from the FSIS Sybase data warehouse.

Results: For NRB, Y1RB and Y2RB, 9369, 13,779 and 6,672 broiler samples and 407, 3806 and 423 turkey samples were collected, respectively. The percentage-positive *Salmonella* decreased from 13.4% in NRB to 8.2% in Y2RB for broilers, and from 10.1% in NRB to 7.1% in Y2RB for turkeys. On average, 4% of broiler samples had HHS *Salmonella* over the three years examined; for turkey, the percentage of such serotypes decreased from 8.1% in NRB to 4.7% in Y2RB. By the end of Y2RB, 83% of broiler establishments and 92% of turkey establishments were in category 1.

Significance: These data suggest the introduction of this initiative had a positive impact on the reduction of *Salmonella* positive in broilers and turkeys.

T6-06 Prevalence of *Salmonella* Species on Marinated Chicken Skin
DSC

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Introduction: Marination of meat products is widely done as it not only adds flavor for increased consumer acceptance but has been also reported to potentially enhance the safety and shelf life of meats. Poultry meat is known to harbor different spoilage and pathogenic bacteria such as, *Salmonella*, *Campylobacter*, *Pseudomonas* and *Listeria* spp.

Purpose: A series of experiments were conducted to determine the effect of teriyaki and lemon pepper marinades on the persistence of multiple strains of nalidixic acid resistant *Salmonella* spp.

Methods: Nalidixic acid resistant *Salmonella* (Typhimurium, Heidelberg, and Seftenberg) cultures were developed by inoculating BHI broth and incubating for 24 h at 37°C; subjecting strains to increasing concentrations of nalidixic acid in XLT4. As a result, (a) *S.* Typhimurium and *S.* Heidelberg resistant to 60 µgm of nalidixic acid, (b) *S.* Seftenberg resistant to

35 µgm of nalidixic acid were obtained. These were inoculated onto chicken skin at levels varying from 0.6 to 3.14 log CFU/g in a 12-well titer plate and placed under a biosafety hood for 30 minutes to allow bacterial attachment followed by marination with teriyaki or lemon pepper marinades respectively. Marinated samples were stored at room temperature (25°C) for up to 32 h. Samples were removed from the marinades at 0, 4, 8, 16, 24 and 32 h and plated in duplicates onto XLT4 followed by incubation at 37°C for 24 h. Experiments were analyzed using a two-way ANOVA to determine significance ($P < 0.05$) of marinades over time.

Results: Prevalence was significantly reduced ($P < 0.05$) by teriyaki marinade on all levels of inoculum whereas no significant effect ($P > 0.05$) was observed for the lemon pepper marinade. Prevalence of *Salmonella* spp. on chicken skin inoculated with 3.14 log CFU/g and marinated with lemon pepper was 100% for all three strains throughout the 32 h storage period. With lemon pepper, prevalence of *S.* Heidelberg and *S.* Seftenberg reduced when inoculated with upto 2.02 log CFU/g during the first 8 h of storage following which it increased to 100% whereas, the prevalence of *S.* Typhimurium started to decrease after 8 h of marination with lemon pepper.

Significance: Marination of chicken skin greatly helped in the reduction of prevalence of *Salmonella* spp. at 25°C for up to 32 h indicating antimicrobial effects in addition to its potential to enhance shelf life of poultry and meat products.

T6-07 The FSIS Routine Risk-based *Listeria monocytogenes* (RLm) Sampling Program

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Introduction: The US Dept. of Agriculture (USDA) Food Safety and Inspection Service (FSIS) samples for *Listeria monocytogenes* (Lm) in establishments that produce post-lethality exposed Ready-to-Eat (RTE) meat and poultry products through the Routine Risk Based Lm (RLm) Sampling Program. During an RLm, product, food contact surface and environmental samples are collected, and a Food Safety Assessment (FSA) is performed.

Purpose: To describe results from the RLm sampling program for April, 2006 through December, 2007.

Methods: Establishments were selected by use of a risk-ranking algorithm, and samples were collected in units proportional to establishment size, consisting of 10 contact surface, 5 environmental and 3 product samples. Samples were analyzed by FSIS laboratories, using methods in the Microbiological Laboratory Guidebook (MLG).

Results: A total of 9,485 samples were collected from 190 establishments. Overall, 67/9,485 (0.71%) samples tested positive, with 2/1,542 (0.13%) product, 16/5,189 (0.31%) contact surface, and 49/2,754 (1.78%) environmental samples testing positive. For the 190 establishments, 33 (17.4%) had at least one positive environmental sample, while 10 (5.3%) had at least one positive food contact surface sample. Drains, wheels and floors, along with equipment blades, knives and scales, were the most common sources of environmental and food contact surface positives, respectively. Pulsed-field gel electrophoresis (PFGE) subtyping yielded 41 patterns among 69 isolates tested. One matching PFGE subtype was obtained from a product and a food contact surface from the same establishment, demonstrating possible cross contamination.

Significance: Percentages of positives were low (< 2%). The highest level of positives was seen in environmental samples, followed by food contact surface and product samples. Identifying sampling sites most likely to test positive and comparing PFGE patterns was useful in identifying possible routes of cross contamination. FSIS uses RLm data to identify contamination of food contact surfaces and production environments and to take regulatory action before contaminated product reaches the marketplace.

T6-08 Thermal Resistance of Thirty Strains of *Salmonella* in Liquid Whole Egg: Are the Optimal Bacterial Strains Being Used in Challenge Studies?

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Introduction: Bacterial inactivation studies are conducted to determine the survival of a target pathogen, such as *Salmonella*, to any number of intervention treatments (e.g., high pressure, antimicrobials, UV light, gamma irradiation, heat, etc.). Resistance of a species of pathogen, such as *Salmonella enterica*, to these treatments can be very large between strains. Proper selection of bacterial strains to be used in inactivation challenge studies is a critical experimental criterion if relevant results are desired.

Purpose: The goal of this study was to screen thirty strains of *Salmonella* for thermal resistance in commercially-processed liquid whole egg (LWE).

Methods: Thirty strains of *Salmonella* were grown in Tryptic Soy Broth at 37°C, with one 24 h transfer, concentrated ten-fold by centrifugation, and resuspended in 0.1% peptone water. Each inoculum was added to LWE and mixed thoroughly, resulting in a final population of ca. 8 log CFU/ml. Inoculated LWE was injected into sterile glass capillary tubes, flame-sealed and heated in a water bath at 58°C for up to 12 min. Capillary tubes were ethanol sanitized, rinsed twice in sterile water and contents were extracted. The LWE was diluted, surface plated onto Tryptic Soy Agar + 0.1% sodium pyruvate and incubated at 37°C for 24 h before colonies were enumerated

Results: Twenty-three of thirty strains were considered heat-sensitive with asymptotic D58 values of less than 1.00 min. The D58 values of three strains (two Enteritidis phage type 8 and one Oranienburg) were 2.11 – 2.61 min. Four more strains (Enteritidis PT 4, PT 13, Oranienburg, and St. Paul) demonstrated similar D58 values of from 1.85 to 1.90 min.

Significance: The four most heat-resistant strains (*Salmonella* Enteritidis PT 8, 8, 13, and Oranienburg) are being used in ongoing ARS/FSIS liquid egg pasteurization studies. These results highlight the importance of carefully selecting optimal bacterial strains to be used in inactivation challenge studies.

T6-09 DSC Thermal Inactivation of *Escherichia coli* O157:H7 in Nonintact Beef Steaks of Different Thickness by Different Cooking Methods and Equipment

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Introduction: Outbreaks of *Escherichia coli* O157:H7 from nonintact beef products have been attributed to inadequate cooking or cooking from the frozen state. Evidence has indicated that when cooking to a given internal temperature, pathogen lethality increases with steak thickness.

Purpose: This study compared thermal inactivation of *E. coli* O157:H7 in non-intact beef steaks of different thickness, cooked to an internal temperature of 65°C by three cooking methods and with different cooking equipment.

Methods: Fresh beef (5% fat) was coarse-ground, and batches (1 kg) were mixed with a composite (50 ml) of rifampicin-resistant *E. coli* O157:H7 (8-strain, 6.4 ± 0.1 log CFU/g) and sodium chloride (0.5%) plus sodium tripolyphosphate (0.25%) solution (50 ml), extruded into casings (10 cm diameter), and placed at -20°C for 6 h. Semi-frozen beef was cut into 1.5, 2.5 and 4.0 cm thick steaks, vacuum-packaged, frozen (-20°C, 48-h), and tempered (4°C, 2.5 h) before cooking. Steaks were double pan-broiled (George Foreman® grill), pan-broiled (Presto® electric skillet and Sanyo® grill) or roasted (Oster® toaster and Magic Chef® kitchen oven) to 65°C. Temperatures of cooking equipment surfaces and meat samples were monitored with thermocouples. Samples were analyzed for survivors on tryptic soy agar plus sodium pyruvate (0.1%, TSAP) and TSAP plus rifampicin (100 µg/ml). Data (two replicates/three samples each) were analyzed using the mixed model of SAS including independent variables and interactions.

Results: The order of pathogen inactivation was roasting (2.0 to 4.2 log CFU/g) > pan-broiling (1.6 to 2.8 log CFU/g) ≥ double pan-broiling (1.2 to 2.3 log CFU/g). Cooking of 4.0 cm thick steaks required a longer time (20 to 65 min; variation due to different cooking equipment) and caused greater ($P < 0.05$) reductions in counts (2.3 to 4.2 log CFU/g) than in thinner samples. The time to reach the target temperature increased in order of George Foreman® grill (3.9 to 19.8 min) < Oster® toaster (11.3 to 45.0 min) < Presto® electric skillet (16.3 to 55.0 min) < Sanyo® grill (14.3 to 65 min) < Magic Chef ® kitchen oven (20.0-63.0 min), with time differences within each equipment related to steak thickness.

Significance: Increased steak thickness allowed greater inactivation of *E. coli* O157:H7, as time to reach the target internal temperature increased. Roasting in a kitchen oven was most effective for pathogen inactivation.

T6-10 Ability of Chemically Softened Water to Rinse Bacteria from the Skin of Processed Broiler

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Introduction: The quality of water used in cleansing operations in commercial poultry processing facilities may have an effect on the efficacy of sanitation operations in these facilities. Water hardness is a characteristic of water that is related to the concentration of calcium and magnesium dissolved in the water. The high concentration of dissolved minerals makes hard water a less effective cleanser than soft water, which contains a lower concentration of minerals; therefore, hard water may also be less effective at removing bacterial contamination from broiler skin.

Purpose: The purpose of this experiment was to examine the ability of hard water that was chemically softened by the addition of various concentrations of potassium citrate to rinse bacteria from the skin of processed broiler chickens.

Methods: Artificial hard water (200 ppm total hardness) was prepared by dissolving 0.38 g calcium chloride and 0.175 g magnesium chloride hexahydrate per liter of distilled water. Hard water was then chemically softened by dissolving 0.0, 1.0, 2.5, or 5.0% (w/v) potassium citrate in the water. Skin samples taken from breasts of broiler carcasses were rinsed five consecutive times in fresh aliquots of chemically softened water, and bacteria in the final skin rinsate were enumerated.

Results: Results indicated that significantly ($P < 0.05$) fewer bacteria were recovered on Eosin Methylene Blue and Campylobacter Agars from skin rinsed in hard water softened with 5.0% citrate than from skin rinsed in water softened with 0.0, 1.0, or 2.5% citrate. Water hardness had no effect on the number of bacteria recovered on *Staphylococcus* or Plate Count Agars, however. In vitro tests indicated that potassium citrate was not bactericidal towards *Escherichia coli*, *Campylobacter jejuni*, or *Staphylococcus simulans* isolates recovered from broiler skin.

Significance: Findings from these experiments indicate that water hardness can play a role in the ability of water to rinse intestinal bacteria from the skin of processed broiler chickens. Since potassium citrate possessed no bactericidal activity, differences in the number of bacteria recovered from the skin was probably due to the differences in the ability of the rinse waters to physically remove bacteria from the skin. Therefore, poultry processors may be able to reduce the number of bacteria on processed poultry by monitoring and controlling the hardness of water used to wash carcasses in processing operations.

T6-11 Same Day Detection of *Escherichia coli* O157:H7 in Large Samplings (3.75 kg) of Fresh or Frozen Raw Ground Beef Using Post Growth Sample Pooling, Re-circulating IMS and Real-time PCR

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Introduction: E. coli O157:H7 has become notorious as a foodborne pathogen because of its low infective dose, its ability to survive for prolonged periods in frozen beef samples and the severity of the disease in vulnerable individuals. Raw ground beef has been implicated as the source of *E. coli* O157:H7 in a significant number of foodborne disease outbreaks and food safety recalls during the past two decades. Detecting the presence of this STEC at low levels in raw beef presents significant challenges to both the food industry and regulatory agencies.

Purpose: This study describes the development and validation of a robust re-circulating IMS (RIMS) method that allows post-growth sample pooling, rapid isolation and detection of *E. coli* O157:H7 from large (10 × 375 g) raw ground

beef samples where initial pathogen levels are in the 1-5 CFU range. The method is applicable to analyzing both fresh and frozen ground beef samples for the presence of *E. coli* O157:H7.

Methods: Fresh or frozen ground beef patties (375 g) were weighed into sterile stomacher bags; one sample in each set of 10 received a low level inoculum of cold stressed nutrient starved *E. coli* O157:H7 (1 to 5 CFU per 3750 g) to mimic very low-level contamination. Pre-warmed Buffered Peptone Water was added and samples were pre-enriched statically at 42°C. Aliquots from each set of 10 pre-enriched, 375 g samples were pooled after pre-enrichment to create single 50 ml samples. Re-circulating immuno-magnetic separation was used to isolate *E. coli* O157 from the pooled samples. A 40-min, 45 cycle real-time PCR was used to detect the presence of *E. coli* O157:H7 in the RIMS eluate. A proportion of the recovered RIMS beads were also plated onto selective agar plates to isolate the target pathogen and confirm the real time PCR results.

Results: The detection of low level *E. coli* O157:H7 contamination in post growth 10 pooled (3750 g) raw ground beef samples was comfortably achieved within a working day using the RIMS pooling strategy linked to real time PCR detection. Recovery of *E. coli* O157:H7 colonies on selective agar plates confirmed the PCR result in all cases.

Significance: The RIMS 10 pooling method for *E. coli* O157:H7 in fresh and frozen ground beef offers high sample throughput, significant cost savings and enhanced pathogen detection. There is no loss of sensitivity, as sample pooling is carried out after pathogen growth but prior to PCR detection. Full traceability of the original sample is maintained until results are confirmed.

T6-12 Use of Dual Electromagnetic Radiation Technology to Reduce *Salmonella* and *Listeria monocytogenes* Risk on Cooked and Packaged Meat Products

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Introduction: Pathogenic bacteria including *Salmonella* and *Listeria* can potentially contaminate Ready-to-Eat meats. These bacteria compromise the safety of our food supply.

Purpose: The objective of this research was to develop and test new low-temperature pasteurization technology for packaged or thermally sensitive foods in order to reduce surface microbial contamination without significant loss in quality. Electromagnetic radiation such as ultraviolet and infrared light has been known for more than 100 years to act as a mutagen at the cellular level. Use of a dual wavelength electromagnetic radiation system including use of infrared and ultraviolet light can achieve maximal bacterial elimination on food product without intolerable heat deposition. A combination of thermal and non-thermal pasteurization treatments were applied in a continuous conveyor system to improve food safety of vacuum packaged Ready-to-Eat food product.

Methods: In this study, a continuous eletromagnetic radiation system with dual wavelength of light was used to treat cooked and vacuum-packaged sausages. A cocktail of six *Salmonella* (*S*. Montevideo, *S*. Senftenberg, *S*. Gaminara, *S*. Heidelberg, *S*. Anatum, and *S*. Typhimurium) or a cocktail of six *Listeria monocytogenes* were surface-inoculated on each sausage link to result in a combined level of 10^9 CFU/cm^2 for *Salmonella* or *Listeria*. The sausages were vacuum-packaged (6 sausages in each 1 lb/package) and held at 4°C for 60 min prior to treatment. Each package was treated in a dual wavelength electromagnetic system with a capacity 160 lb/min at a conveyor speed up to 24 ft/min. *Salmonella* and *Listeria* were enumerated on sausages.

Results: Salmonella and *L. monocytogenes* were both reduced by 3 logs.

Significance: The results from this study are important for any food processor where risk of surface pathogen contaminations is a concern. This research offers a faster, cheaper, and less quality-disruptive alternative to traditional thermal-processing for lowering foodborne pathogen levels.

T7-01 An Innovative Modeling Approach for Food Safety Risk Assessment Research for the Farm-to-Fork Continuum

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Introduction: Quantitative probabilistic microbial risk assessment models (QPRAM) have yet to be applied to fresh fruits and vegetables. Because fresh produce is vulnerable to contamination with pathogens at any point, a farm-to-fork modeling approach is advisable.

Purpose: To develop a QPRAM to characterize the risk of foodborne disease associated with a candidate pathogen-commodity pair. The model was designed specifically to facilitate evaluation of the relative efficacies of potential control strategies, serving as a "virtual laboratory" for FDA to investigate alternative contamination scenarios based upon available data and evaluate the efficacy of different mitigation strategies.

Methods: The initial focus was on *E. coli* O157:H7 and romaine lettuce. The farm-to-fork chain was subdivided into modules of production; harvest; post-harvest unit operations; distribution; and consumption. Specific risk factors were identified for each module. Information obtained through literature review and expert elicitation was used to estimate input values for the risk factors. Inputs focused on the probability of occurrence of each risk factor, and the probability of contamination conditioned on occurrence. A novel Agent-Based Modeling (ABM) framework allowed for explicit modeling of temporal and spatial impacts of risk factors along with potential interactions among modeling agents.

Results: Initial model investigations indicate that animal access to the farm and irrigation are highly significant contributors to contamination of romaine lettuce on farm. Mitigation options that restrict animal access to the farm and avoid specific, low-quality sources of irrigation water were shown to effectively reduce the number of contaminated lettuce heads.

Significance: The approach is relevant to a wide variety of pathogens and food commodities. It will also be instrumental in evaluating the efficacy of food safety policies and quantifying the relative reductions in risk associated with proposed interventions.

T7-02 Assessment of Methods to Verify Standards for Reduction of Risk from Aerosol Transfer of *Escherichia coli* from Composting and Livestock Operations

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Introduction: Audit standards (metrics) for lettuce and leafy greens production in CA and AZ specify a separation of 9 to 122 m from adjacent sources of likely fecal contamination, such as compost facilities and livestock operations. The foundation assumptions and appropriateness of these distances have not been adequately tested in environments relative to production of these commodities.

Purpose: Conduct an assessment of practical methods to establish evidence for transfer and survival of *E. coli* and related bacterial fecal indicators and pathogens (EFIP) in aerosols and airborne particulates to adjacent leafy green crops.

Methods: Aerosol capture was conducted, using an array of six MAS-100Eco™ samplers and combinations of nonselective and selective/differential media. In addition, SKC button samplers with gelatin membranes were evaluated for direct detection of molecular markers of fecal contaminants. Sampling locations, within and beyond the metrics, included compost facilities, large-animal corrals, dairy operations, and adjacent lettuce and spinach research fields. Aerosol source material, environmental, and plant samples were collected and processed for recovery and detection of EFIPs, using conventional culture and PCR.

Results: Aerosol capture of high populations of viable *E. coli* within 9 m and diffuse concentrations at 30.5 m of a feedlot point source was demonstrated but not at 61m or beyond. Detection of pathogenic *E. coli* DNA from aerosol capture was demonstrated at 9 m from a feedlot. Particulate aerosol capture of viable EFIP from a dairy source was not demonstrated over a 64-day period, but culture-confirmed *E. coli* O157:H7 were recovered from adjacent environmental and spinach samples on multiple dates. Capture of water aerosols from a point-source release exceeded 122 m under moderate wind conditions.

Significance: Preliminary assessments support the retention of conservative metrics for crop separation from aerosol sources. Aerosol sampling is generally more limiting than crop sampling to address commodity-specific risks and develop data-based regional standards

T7-03 Risk Assessment of *Campylobacter* Infection Due to Poultry Meat Consumption in Japan

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Introduction: Recently *Campylobacter* has been playing a major role in causing foodborne illnesses in Japan. The reported number of foodborne Campylobacteriosis is ca. 2,400 per year; however, a study estimates that about 1.5 million people suffer from acute gastroenteritis caused by *Campylobacter* every year.

Purpose: Stochastic risk assessment model was developed to estimate the current risk of *Campylobacter* infection due to poultry meat consumption in Japan and possible risk reduction by mitigation measures.

Methods: Input variables were estimated from prevalence and contamination data for *Campylobacter* at farm and retail stages in Japan, or from population survey data for poultry meat consumption and cooking. A dose-response model developed by Health Canada was used to calculate the probability of infection at a given dose of *Campylobacter*. The model was constructed and simulation was run using @RISK.

Results: The simulation results indicated that a Japanese consumer had risk of *Campylobacter* infection with mean value of 1.35 times per year, and the most influential input was the frequency of raw poultry meat consumption. Reducing contamination in the farms had limited risk reduction effect compared to preventing cross contamination during cooking and restricting raw meat consumption, however, when combined with logistic slaughtering after testing adult flocks, decreased farm contamination was the most effective means of risk reduction in the human population, even if a certain fraction of people prefer raw poultry meat.

Significance: A newly developed stochastic model allowed comparison of possible control measures to be applied at various stages of thefood chain of poultry meat in terms of human campylobacteriosis reduction.

T7-04 A Mathematical Risk Model for *Escherichia coli* O157:H7 Cross-contamination of Lettuce during Processing

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Introduction: Consumption of fresh-cut lettuce has resulted in recent outbreaks of *Escherichia coli* O157:H7 infection with the pathogen clearly able to survive throughout the farm-to-fork continuum. In addition to product initially contaminated in the field, this pathogen can also be transferred to uncontaminated product during harvesting, processing and packaging.

Purpose: A risk assessment approach was taken to determine the extent of *E. coli* O157:H7 contamination in fresh-cut bagged lettuce leaving the processing plant based on transfer data obtained in our laboratory and to estimate the risk to consumers by incorporating data from other sources.

Methods: A probabilistic model was constructed in Excel to account for *E. coli* O157:H7 cross contamination when contaminated lettuce enters the processing line. Simulation of the model was performed using @Risk Palisade© Software, providing an estimate of concentration and prevalence in the final bags of product.

Results: The model was satisfactorily validated based on Standard Error of Prediction, which ranged from 0.00-35%. ANOVA performed on simulated data revealed that the initial level in the contaminated batch (S1: 0.01 CFU/g; S2: 1 CFU/g; S3: 100 CFU/g) did not influence ($P = 0.4$) the *E. coli* O157:H7 levels in bags derived from cross contamination. In addition,

significantly different ($P = 0.001$) prevalences were observed at the different levels simulated (S1; S2 and S3). At the lowest contamination level (0.01 CFU/g), bags were cross-contaminated sporadically, resulting in very low *E. coli* O157:H7 populations (≤ 2 CFU/bag) and prevalence levels (< 1%).

Significance: Results obtained for the lowest contamination levels (S1) suggest that most sampling plans will not effectively detect *E. coli* O157:H7 during production. Hence, in the absence of an *E. coli* kill step, diligent application of sanitizers to the equipment and wash water is recommended to reduce cross contamination during processing.

T7-05 Analyzing the Power and Error of *Listeria monocytogenes* Growth Challenge Studies

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Introduction: Domestic and international food safety policy developments have spurred interest in the design and interpretation of experimental growth challenge studies to determine whether Ready-to-Eat (RTE) foods are able to support growth of *L. monocytogenes*. Current challenge study protocols apply a fixed acceptance criteria value (i.e., a difference less than a X log increase) to distinguish real growth from quantitative measurement uncertainty but differ markedly in terms of experimental design and the acceptance criteria under which a RTE food is determined not to support *L. monocytogenes* growth.

Purpose: The objective of the study is to analyze that statistical power and type I (false positive) error probability (α) of *L. monocytogenes* challenge study protocols and alternative acceptance criteria.

Methods: Conventional statistical power analysis and Monte Carlo simulation methods are used to analyze the power and type I error of tests of the no-growth hypothesis under alternative true levels of growth.

Results: The current protocols differ substantially with respect to the probability of incorrectly determining that growth occurs and the statistical power to detect growth if it does occur. However, applying any fixed acceptance criteria exceedance value (e.g., less than a 0.5 log or 1 log increase) to distinguish real growth from quantitative measurement uncertainty over different experimental designs and/or measurement uncertainty values implies highly inconsistent type I error probabilities. None of the *L. monocytogenes* growth challenge study designs currently being considered are likely to provide an F-test with $\alpha = 0.05$ and power ≥ 0.8 to detect a 1 log increase in mean concentration over the entire range of measurement uncertainty values for enumeration of *L. monocytogenes* reported in food samples in a validation study of ISO Method 11290-2.

Significance: Satisfying the conventional experimental design criteria would require a larger sample size, lower measurement uncertainty, or both.

T7-06 DSC Geographic Information Systems Mapping of Foodservice Health Code Violations to Assess Risks for Foodborne Illness in Populations of Different Socioeconomic Status

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Introduction: Some data suggests that the incidence of foodborne illness may be associated with an individual's socioeconomic status (SES), but it is unknown why this may occur. In recent years, the ability to associate environmental conditions with geographic locations has improved greatly using Geographic Information Systems (GIS) which allows for the integration and analysis of geographic and tabular data for detection of environmental gradients. Because low SES is often associated with geographic regions, this research investigated whether GIS technology could be used to correlate health code critical violation rates with SES.

Purpose: This research used GIS technology to investigate whether community socioeconomic status relates to access to safe food as measured by foodservice critical health code violations.

Methods: Publically available health inspection records documenting critical health code violations for 13,485 foodservice locations, collected between 2005 and 2008 in Philadelphia, PA, were analyzed. Using an overlay analysis through GIS, critical violations were plotted over census tracts in Philadelphia, PA. Census tracts (n = 368) were categorized into one of 5 SES categories based on poverty level.

Results: Average proportions of population below poverty in different categories were significantly different and ranged from 5.8% to 52.6%. The average rate of critical violations for all foodservice facilities was 0.67 per inspection. 52% of the facilities had zero critical violations. Rates of violations in poverty groups were: 0.83 ± 1.43 (lowest poverty), 0.64 ± 1.25, 0.62 ± 1.02, 0.63 ± 1.00, and 0.69 ± 1.02 (highest poverty) and the differences were found to be statistically significant. These results indicate an association of higher rates of violations with lowest poverty rates.

Significance: This study demonstrates the ability to utilize GIS technology to correlate possible food safety risks with geographic data related to demographics of populations.

T7-07 DSC Defining Strain-specific Infective Doses Required for the Establishment of a Systemic *Listeria monocytogenes* Infection

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Introduction: Listeria monocytogenes is a human foodborne pathogen that may cause severe invasive disease in susceptible individuals. *L. monocytogenes* utilizes the virulence factor Internalin A (InlA; encoded by *inlA*) to induce its uptake by non-professional phagocytes that express the human isoform of E-cadherin. At least 18 naturally occurring mutations leading to a premature stop codon (PMSC) in *inlA* have been identified worldwide and these mutations have been shown to be causally associated with attenuated mammalian virulence.

Purpose: The purpose of this study was to evaluate the hypothesis that the dose-response relationship varies by *L. monocytogenes* subtype. Guinea pig infection experiments were conducted to define strain-specific infective doses for a fully virulent *L. monocytogenes* outbreak strain and a naturally occurring virulence-attenuated strain carrying the most common *inlA* PMSC mutation.

Methods: Juvenile male guinea pigs were intragastrically infected with a range of doses of either (1) a fully-virulent serotype 4b outbreak strain from a patient associated with the 1998 U.S. listeriosis outbreak or (2) a virulence-attenuated serotype 1/2a food isolate carrying a PMSC in *inlA*. Recovery of *L. monocytogenes* from four internal organs (i.e., liver, spleen, mesenteric lymph nodes, and ileum) was used to define infection status.

Results: The outbreak strain was recovered from 85% of organs (based on all four organs) from guinea pigs infected with a dose of 1×10^8 CFU; 30.5% of organs from animals infected with 1×10^7 CFU; 25% of organs from animals infected with 1×10^6 CFU, and no organs from animals infected at a dose of 1×10^5 CFU. The naturally occurring food isolate carrying a virulence-attenuating PMSC mutation in *inlA* was recovered from 100% of organs from guinea pigs infected at 1×10^{10} CFU, 22.5% of organs from animals infected at 1×10^8 CFU, and 20% of organs from animals infected at 1×10^7 CFU. A fisher's exact test showed that the strain carrying a PMSC in *inlA* was recovered from fewer organs from guinea pigs infected with a dose of 1×10^8 ($P < 0.0001$) and 1×10^7 ($P = 0.0016$) as compared to animals infected at the same doses of the outbreak strain.

Significance: This work supports that the dose-response relationship is strain-specific, and results from this work will facilitate the development of an accurate dose response model for *L. monocytogenes* with and without PMSCs in *inlA* and provide critical data needed for enhancement of future *L. monocytogenes* risk assessments and regulatory initiatives.

T7-08 Impact pf Predator-prey Dynamics in Reducing Seafood Spoilage Bacteria

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Introduction: Food biopreservation has been one of the dominant topics in microbiological research for a decade but commercial applications are still pending. *B. dellovibrionaceae* (BD) are unique, predatory, endoparasitic, Gram-negative bacteria and potential biological control agents. A halophilic species of BD was isolated from fourteen coastal sea water sites around New Zealand.

Purpose: Increased understanding of the unique predatory mechanisms of BD could create a new and potentially fertile field to control harmful Gram-negative bacteria. To pursue this, there is a need to isolate and characterise BD in New Zealand and to investigate the potential application of BD as a biopreservative agent.

Methods: BD isolates were characterized using proven characterization techniques, including general, microscopic and molecular techniques.The in-vitro efficacy of BD was assessed against seafood spoilage bacteria. The biopreservation capability of BD to extend the shelf life of king salmon was evaluated.

Results: The in vitro efficacy of BD was assessed against foodborne pathogenic and spoilage bacteria (particularly *Photobacterium phosphoreum*). Log reductions of *P. phosphoreum* and some other Gram-negative bacteria ranged from 4.5 to 4.8 after 9 h of incubation at 25°C. BD was effective in reducing the numbers of *P. phosphoreum* at pH 5.5 to 8.5 and salinity 0.9 to 4.5% (w/v). A significant interaction was observed between the prey and predator concentrations and nutrient concentration. Prey concentrations were observed to be the most vital factor in predation and the most favourable predation conditions were at a prey concentration of ~8 log CFU/mL, together with a predator concentration of 3 to 7 log plaque forming units (PFU)/mL and a prey : predator ratio of > 5.0. The thresholds of the prey and predator concentrations for predation were observed to be 3.7 log CFU/mL and 3.9 log PFU/mL, respectively. The biopreservation capability of BD in extending the shelf life of king salmon was evaluated, a significant effect was observed at 20°C but not at 10°C.

Significance: It is concluded that the possibility of industrial application of BD as an agent in the control of Gram-negative spoilage and containment organisms has been demonstrated in this study. However, the benefits of further investigations into the potential of BD in the fresh fish industry will depend on finding BD that can have a beneficial effect under refrigerated conditions.

T7-09 Potential for Microbiological Spoilage in High-pressure Processed Food

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Introduction: High Pressure (HP) processed products preserve the organoleptic properties of fresh products by providing a microbiological reduction treatment without the negatively associated thermal quality changes. However, all packaged products undergoing a microbial reduction process are subject to potential leaker spoilage

Purpose: The study sought to assess the potential risk of leaker spoilage by determining the number of microorganisms in the HP transmission fluid, their survival through the HP process, and the potential for leakage into the product packs.

Methods: Samples of the transmission fluids and surfaces in the product contact zone were taken from a number of commercial and research HP units to assess microbial loading. Survival of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was assessed, in suspension (planktonic) and when grown as biofilms on stainless steel coupons, under a range of pressures (300MPa to 600MPa) for 3 min. The migration of microorganisms from the HP unit transmission fluid into simulated food packs was determined by processing pouches containing sterile Growth Medium, placed inside pouches containing *Staphylococcus epidermidis* of a predetermined genetic fingerprint. After the treatment with HP (300MPa to 600MPa) the pouches were incubated and from each sample which showed microorganism growth (turbidity), microorganisms were isolated, purified and ribotyped.

Results: The TVC of the transmission fluids of the commercial units ranged from 10^0 to 10^3 CFU/ml whilst the TVC on product contact surfaces ranged from 10^0 to 10^5 CFU/cm^2. Highest counts were associated with the piston sealing arrangement. Differential survival with pressure was observed with a 6.3 log reduction for *P. aeruginosa* and a 0.04 log

reduction for *S. aureus* at 330MPa. There was no survival of either organism at 600MP though microorganisms grown as biofilms were more resistant than their planktonic counterparts. A number of pouches showed microbial growth at pressures up to 500MPa. One pouch contained the same riboprint as the test suspension whilst the other 'spoilage' microorganisms were identified as typical skin flora.

Significance: A potential risk of leaker spoilage for HP processed products was demonstrated, which can be controlled by minimizing contamination in the HP units and via more hygienic pack handling.

T7-10 Inactivation of Bacterial Spores in Tomato Sauce by High Hydrostatic Pressure DSC

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Introduction: Bacterial spore formers, like *Alicyclobacillus acidoterrestris* and *Bacillus coagulans*, can cause flat-sour type of spoilage in heat-processed tomato products. *B. coagulans* is moderately acidophilic, being able to grow down to a pH of about 4.0, while *A. acidoterrestris* can even grow at a pH as low as 2.5. Since the spores of both organisms are both heat and acid resistant, they can survive the commercial heating processes applied to tomato products.

Purpose: The objective of this work was to study the germination and inactivation of *A. acidoterrestris* and *B. coagulans* spores in tomato sauce by high hydrostatic pressure treatment at temperatures up to 60°C.

Methods: Spore suspensions of *A. acidoterrestris* ATCC49025 and *B. coagulans* ATCC7050 (approximately 10^7 CFU ml^{-1}) in citric acid (0,02 M, pH 3.5 to 5.0) or tomato sauce (pH 4.2 or 5.0) were subjected to pressure treatments at 100 to 800 MPa (5 to 60°C, 10 min). After processing, spore inactivation and germination was determined by plating the suspensions either directly, or after an additional heat treatment to kill the germinated spores. All experiments were conducted in triplicate and data are expressed as mean values ± standard errors of mean.

Results: In general, little or no inactivation of *A. acidoterrestris* spores after a HHP treatment (100 to 800 MPa, 10 min) was noticed at all temperatures tested. However, treatment at low pressures (< 400 MPa, optimum of about 200 MPa) caused germination of *A. acidoterrestris* spores. The proportion of germinated spores increased with increasing process temperature, upto 99%. Spores of *B. coagulans* also germinated upon high pressure treatment, but showed more germination as pressure increased.

Significance: The data show that germination of *A. acidoterrestris* and *B. coagulans* spores can be induced by high hydrostatic pressure treatment in an acidic environment. In contrast, spores of non-acidophiles, like *B. subtilis*, cannot be pressure-germinated in acidic conditions. We anticipate that it will be possible to inactivate *A. acidoterrestris* and *B. coagulans* spores by applying a two-step process consisting of a mild high pressure treatment to induce germination, followed by a mild heat treatment or a more elevated pressure treatment to kill the germinated spores.

T7-11 Membrane Damage and Microbial Inactivation by UV-light and Radio Frequency Electric Fields Processing of Apple Juice

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Introduction: Radio frequency electric fields (RFEF) and UV-light treatments have been reported to inactivate bacteria in liquid foods. However, information on the efficacy of bacterial inactivation by combined treatments of RFEF and UV-light technologies is limited.

Purpose: In this study, we investigated the relationship between cell injury and inactivation of *Escherichia coli* K-12 in apple juice treated with a combination of RFEF and UV-light.

Methods: Apple juice purchased from a wholesale distributor was inoculated with *Escherichia coli* K-12 at 7.8 log CFU/ml, processed with a laboratory scale RFEF at 20 kHz, 15 kV/cm for 170 µs at a flow rate of 540 ml/min followed by UV-light treatment at 40C. Treated samples were analyzed for leakage of UV-absorbing substances as a function of membrane damage and were plated (0.1 ml) on Sorbitol MacConkey Agar (SMAC) and Trypticase Soy Agar (TSA) plates to determine viability loss and percent injury.

Results: UV-light treatment alone caused 6.5 log reduction of *E. coli* in apple juice while RFEF caused only 1.5 log reduction. Percent injury caused by RFEF and UV-light processing alone averaged 95% and < 1%, respectively. A combination of the two processing treatments did not increase cell injury or leakage of UV-substances. Similarly, the number of viability loss determined was not significantly ($P < 0.05$) different than RFEF or UV-light treatment alone. However, the UV-absorbing substances determined in apple juice treated with RFEF was significantly ($P > 0.05$) different than UV-light treated samples.

Significance: The results of this study suggest that RFEF treatment causes more injury to the bacterial cells leading to more cellular leakage of UV-substances than UV-light treatment. Also, that the effect of the two processing treatment combination was not synergetic.

T7-12 Assessment and Modelling of the Microbial Spoilage of Four Traditional Greek Appetizers

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Introduction: Traditional Greek appetizers are emulsified foods made of vegetables, bread, legume, cheese, and sometimes mayonnaise. Their shelf life ranges from 2 to 3 months under refrigeration and relies on acetic (vinegar) or citric (lemon) acid (pH 3.6-3.9) and NaCl (3-7%); however, in tropical climates, where temperatures during distribution and household refrigerators may often exceed 5 or even 10°C, remarkable microbial growth may occur, despite their intrinsic stability. Therefore, assessment/prediction of their shelf life via a relevant decision support tool, based on the time-temperature exposure of the products in the market, is of high value.

Purpose: The objectives of the present study were: (1) to identify the specific spoilage organisms (SSO) and spoilage level (SL) of 4 commercial traditional Greek appetizers namely, pepper-salad, aubergine salad, legume salad and garlic-based salad; and, (2) to develop and validate predictive models for the growth of SSOs.

Methods: Commercial packages of salads were supplied by a Greek Industry and stored at 4, 10, 15 and 20°C until the occurr-ence of spoilage. Enumeration of total viable counts, lactic acid bacteria (LAB), and yeasts/moulds were carried out at regular time intervals, in parallel to measurements of titratable acidity, pH, aw, organic acids (HPLC) and sensory analysis by industrial panels. The growth of LAB was modelled through primary models, and their kinetic parameters were further modelled as a function of temperature with square root models.

Results: Different aciduric LAB (e.g. *Lactobacillus plantarum*), determined by SDS-PAGE, dominated the spoilage flora of these products at different temperatures. Products were spoiled when LAB exceeded 8 log CFU/g, causing marked increase in titratable acidity (e.g. from 0.6 ml initially to 2.9 ml of 0.1N NaOH at the occurrence of spoilage), production of acetic acid, CO_2 and ethanol and appearance of sour taste. Based on sensory analysis, spoilage occurred at 25, 50, 70 and 90 days of storage at 20, 15, 10 and 4°C, respectively. The slope of maximum specific growth rate of LAB versus temperatures from 15 to 20°C was different from that versus 4 to 10°C, possibly due to the different LAB dominating at each temperature range. Validation of models under dynamic temperature conditions showed satisfactory performance for all 4 salads.

Significance: The results suggest that traditional Greek appetizers are prone to spoilage by LAB and predictive models may assist in monitoring of their microbial quality along the food-supply chain. Therefore, such decision-support systems may effectively assist in the management of products distribution.

T8-01 DSC Effect of Antimicrobial Sanitizers and High Power Ultrasound on Murine Norovirus on Romaine Lettuce

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Introduction: Human noroviruses (NoV) are the leading cause of foodborne viral gastroenteritis worldwide. Foods commonly implicated in NoV outbreaks are those that have undergone minimal processing, such as fresh produce. Sodium hypochlorite and peroxyacetic acid are antimicrobial sanitizers used in the fresh produce industry, but their effect on NoV has not been well studied. High power ultrasound (HPU) is a technology that has the potential to improve the antimicrobial effects of these sanitizers on contaminated produce items.

Purpose: To investigate the effect of sodium hypochlorite and peroxyacetic acid in combination with HPU on murine norovirus (MNV-1) inoculated on lettuce.

Methods: Romaine lettuce leaves (5 cm^2) were spot-inoculated with 50 µl MNV-1 (approximately 8-log PFU/ml). Once dried, inoculated leaves were immersed in 25 or 100 ppm sodium hypochlorite (pH 7.0) or 80 ppm peroxyacetic acid, with or without HPU, for 1 min at 10°C. Virions were recovered from leaves by vortexing in 3% beef extract, pH 8.0, and quantified by plaque assay in RAW 264.7 cells.

Results: Treatment with 25 or 100 ppm sodium hypochlorite reduced infectious MNV-1 titer by 1.66 log and 2.27 log units, respectively, and the addition of HPU during treatment increased reduction to 2.65 log and 3.14 log units, respectively. Similar reductions were recorded in treatments with 80 ppm peroxyacetic acid, with a 2.13 log reduction achieved alone and a 2.88 log reduction when combined with HPU. Further investigation into the ratio of lettuce leaves to sodium hypochlorite volume indicated that the efficacy of the combined treatment with HPU was unaffected between 1:500 and 1:50 (w/v).

Significance: The results indicate that HPU can improve the antimicrobial effect of either of two sanitizers tested on MNV-1 on lettuce.

T8-02 DSC Thermal Inactivation of *Escherichia coli* O157:H7 at Different Depths of Panbroiled and Roasted Non-intact Steaks

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Introduction: Escherichia coli O157:H7 transferred to internal muscle tissue during tenderization is a potential cause of foodborne illness if the meat is undercooked.

Purpose: This study evaluated thermal inactivation of *E. coli* O157:H7 at different depths in non-intact steaks cooked by panbroiling or roasting to a sublethal temperature.

Methods: Fresh beef (*Semitendinosus*; 3% fat) was cut (3 mm thickness) and eight slices were stacked on each other and tenderized (4.8 blades/cm^2), using a Supertendermatic™ hand-held tenderizer to form 50 × 60 × 24 mm non-intact steaks. Steaks were inoculated with rifampicin-resistant *E. coli* O157:H7 (8 strains; 3.7 log CFU/cm^2) on the surface (0 mm) or between the slices at a depth of 3, 6, 9 or 12 mm. Each steak was vacuum-packaged, stored (4°C, 12 h) and cooked by panbroiling, with turning every 2 min, or roasting at 149°C, to a geometric center temperature of 60°C. The temperature of the geometric center and depth of inoculation (3, 6, 9 or 12 mm) of each steak was recorded with thermocouples. After cooking, a core sample (1.61 cm^2 × 24 mm) was excised from the center of each steak and analyzed for *E. coli* O157:H7 on Tryptic Soy Agar containing 0.1% sodium pyruvate and 100 µg/ml rifampicin. The experiment was repeated twice, with six samples per replicate. Data were analyzed by cooking method as a single factor (inoculation depth) ANOVA in Proc Mixed of SAS. Means were separated using a *F*-protected *t*-test.

Results: Cooking times to reach a geometric center temperature of 60°C by panbroiling and roasting were 11.5 ± 1.2 and 26.2 ± 4.3 min, respectively. Higher internal temperatures were observed during cooking by panbroiling than roasting at 3 (88.0 ± 7.3 vs 63.4 ± 4.8°C), 6 (73.7 ± 8.9 vs 59.6 ± 10.0°C) and 9 (64.5 ± 4.8 vs 60.5 ± 2.1°C) mm depths of inoculation. Surviving pathogen populations increased ($P < 0.05$) from 0.3 ± 0.6 log CFU/cm^2 at 0 mm to 2.5 ± 0.6 log CFU/cm^2 at 12

mm depth of inoculation in steaks cooked by panbroiling. For samples cooked by roasting, although a similar increasing trend in pathogen populations was observed, thermal destruction of *E. coli* O157:H7 was less and differences in pathogen populations were not significant ($P \geq 0.05$) at each depth of inoculation.

Significance: There was more thermal destruction throughout, higher internal cooking temperatures and shorter cooking times when undercooking non-intact steaks by panbroiling than roasting to the same internal temperature.

T8-03 *Listeria monocytogenes* Outbreak Strains Demonstrate Differences in Invasion Efficiency and Other Virulence Associated Characteristics

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Introduction: Listeria monocytogenes is a facultative intracellular pathogen that can cause a severe invasive foodborne disease and occasional large outbreaks. During infection, bacteria express the virulence gene *inlA*, which encodes a key protein required for efficient cell invasion. While a number of molecular subtyping studies on outbreak strains have been reported, few comprehensive data sets on the invasiveness and other virulence associated characteristics of outbreak strains are available.

Purpose: To (1) more comprehensively examine and compare invasion efficiency and other virulence associated phenotypes among *Listeria monocytogenes* strains from human listeriosis outbreaks, and to (2) probe the possible contribution of *inlA* sequence and/or expression differences, as well as differences in flagellar motility, to variable invasion phenotypes.

Methods: Fifteen *L. monocytogenes* strains from human listeriosis outbreaks were characterized for invasiveness in Caco-2 human intestinal epithelial and HepG2 human hepatic cells using bacteria grown under two conditions (30°C without shaking and 37°C with shaking). DNA sequencing and phylogenetic analysis of *inlA* were performed on all strains and a subset of four strains demonstrating attenuated invasiveness was further characterized for *inlA* expression using qRT-PCR and for flagellar motility by swarming assays.

Results: The outbreak strains showed significant variation in their ability to invade Caco-2 cells ($P < 0.05$) and HepG2 ($P < 0.001$) cells under both bacterial growth conditions, with four strains showing notable invasion attenuation. DNA sequencing of *inlA* showed that none of these four strains contained known virulence-attenuating mutations. Phylogenetic analyses of *inlA* clustered the four outbreak strains showing reduced invasion efficiency with two outbreak strains demonstrating high invasion efficiency. Two strains with low invasion efficiency showed significantly reduced *inlA* expression ($P < 0.05$), while the two other strains with low invasion efficiency showed reduced swarming ($P < 0.05$).

Significance: Our data show that *L. monocytogenes* strains from human listeriosis outbreaks demonstrate significant variation in their ability to invade human cells, and that different mechanisms may contribute to reduced invasion efficiency. Thus, associations between a few specific strains and human outbreaks likely involve phenotypic characteristics other than enhanced invasion efficiency, including possibly an enhanced ability to persist and grow in food and food associated environments.

T8-04 Detection and Quantification of Rota Virus (RV) from Fresh Produce by Real-time RT-PCR and Cell Culture DSC

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Introduction: Viral foodbornes disease caused by enteric viruses such as rotaviruses haves a significant public health concern. These viruses contaminated fresh fruit and vegetables and these foods may act as vehicles. Since only few viral particles may cause disease, detection of low concentration of viruses is crucial for control of viral foodborne disease.

Purpose: The purpose of this study was to evaluate and compare different elution and concentration methods for optimization of virus detection method, using real-time RT-PCR and cell culture techniques.

Methods: Leafy vegetable samples (lettuce, Chinese cabbage) were artificially inoculated with rotavirus. Viruses were extracted from the lettuce by two different elution buffers, buffer A [100 mM Tris-HCl, 50 mM glycine, 3% beef extract pH 9.5] and buffer B [250 mM Threonine, 300 mM NaCl pH 9.5], and extracted viruses were concentrated by ultrafiltration and PEG precipitation sequentially. To determine infectivity of the viruses, viruses recovered from the samples were infected to the MA-104 cell, and integrated cell culture real-time RT-PCR was performed at 48, 72, 96, 120, 144, 168 h post infection.

Results: Elution buffer A was more efficient at extracting the virus from produce samples than buffer B. The sensitivity of real-time RT-PCR method was markedly improved when the virus was concentrated by the ultrafiltration method. When the viruses were eluted and concentrated by buffer A and ultrafiltration, respectively, average recoveries were ca. 67.69% and the detection limit was 6.8×10 RNA copies/g. When the viruses extracted and concentrated from samples were infected to MA-104 cell, a cytopathic effect was observed at 3 to 5 days post infection, with a rapid decrease of Ct value.

Significance: The optimized detection method evaluated in this study can be useful for rapid and reliable detection of RV in fresh produce products and can be applied for detection of other foodborne viruses.

T8-05 Phage Therapy Reduces Lairage-induced Increases in *Salmonella* Colonization in Market Weight Pigs

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Introduction: Contamination of meat and meat products with foodborne pathogens usually results from the carcass coming in contact with the feces of an infected animal during processing. In the case of *Salmonella*, several recent studies have reported that pigs become rapidly infected with the organism during transport and lairage due to contaminated trailers and holding pens. These infections serve to increase the likelihood of carcass contamination by amplifying the number of bacteria that enter the processing facility.

Purpose: We conducted a series of experiments to test whether phage therapy could be used to counteract *Salmonella* infections at this critical pre-processing period.

Methods: Fourteen anti-*Salmonella* phage were isolated from wastewater treatment facilities and characterized by electron microscopy. In preliminary experiments, microencapsulated phage were co-administered with *Salmonella enterica* Typhimurium to 4 to 5 week-old pigs (n = 8 per group). In the main experiment designed to mimic a production setting, microencapsulated phage were administered to market weight pigs (n = 8 per group in three replicates) prior to their comingling with *Salmonella enterica* Typhimurium infected pigs in a contaminated pen.

Results: Treating the small pigs with the phage cocktail at the time of inoculation with *Salmonella enterica* Typhimurium reduced the extent of *Salmonella* colonization by 99.0 to 99.9% (2 to 3 log growth) in the tonsils, ileum, and cecum as compared to mock-treated pigs. Under the production-like setting, treatment of market weight pigs with the anti-*Salmonella* phage cocktail significantly reduced cecal *Salmonella* counts (95%, $P < .05$) while also showing a strong tendency to reduce ileal *Salmonella* counts (90%, $P = .06$). In vitro studies demonstrated that the phage cocktail was also effective at lysing several non-Typhimurium *Salmonella* serovars.

Significance: Taken together, these data indicate that phage therapy can be used as an effective anti-*Salmonella* intervention strategy to prevent lairage-associated increases in *Salmonella* colonization in pigs.

T8-06 Comparison of the Microbiological Profiles of Conventionally-raised and Grass-fed Beef Samples

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Introduction: Grass-fed beef is often promoted as a safer alternative to conventionally-raised (i.e., grain-fed) beef due to the effects that the forage diet and the exclusion of antibiotics in the feed may have on intestinal pathogens. Data either supporting or refuting this claim, however, are scarce.

Purpose: We conducted several experiments to compare the microbiological profiles of conventionally-raised (CR) and grass-fed (GF) beef samples.

Methods: CR and GF beef samples (n = 35) were collected from retail markets throughout Indiana. Individual samples were screened for the presence of general coliforms, generic *E. coli*, *Enterococcus*, *E. coli* O157:H7 and *Salmonella*. Individual *E. coli* isolates from positive samples were tested for resistance to 17 antibiotics and antimicrobials commonly used in either human medicine, animal agriculture or both.

Results: Overall, CR and GF beef samples had similar microbiological profiles. Coliforms were isolated from 94% (avg. 3.2×10^3 CFU/mL rinsate) of CR beef samples and 89% (avg. 1.4×10^4 CFU/mL rinsate) of GF beef samples. Likewise, *Enterococcus* was isolated from 71% of CR beef samples and 83% of GF beef samples, while *E. coli* was isolated from 65% of CR beef samples and 44% of GF beef samples. Neither *Salmonella* nor *E. coli* O157:H7 was isolated from either group. CR beef and GF beef had similar antibiotic resistance profiles. *E. coli* isolates from both groups were most commonly resistant to sulfisoxazole (53% and 50% in CR and GF beef, respectively). There were low levels of multiple antibiotic resistance in both groups: 5% in CR beef vs. 14% in GF beef. No regularly occurring multiple antibiotic resistance patterns were detected in either group.

Significance: Taken together, these data indicate that retail beef samples from conventionally-raised cattle and grass-fed cattle have similar microbiological profiles in terms of contamination with general coliforms, *E. coli*, *Enterococcus*, and antibiotic resistance bacteria.

T8-07 DSC *Listeria monocytogenes* Epidemic Clone Strains and Strains Harboring Virulence Attenuating Mutations in *inlA* Show Evidence of Niche Adaptation

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Introduction: Listeria monocytogenes is a bacterial foodborne pathogen that causes a serious disease, listeriosis, in susceptible populations. Epidemic clone (EC) strains have been linked to the majority of listeriosis outbreaks worldwide and are overrepresented among sporadic listeriosis cases in the United States. On the other hand, over 30% of isolates from food harbor premature stop codon (PMSC) mutations in the key virulence gene *inlA*, which have been shown to be responsible for attenuated invasion in human cell lines and guinea pigs. Internalin A (InlA) binds the human and guinea pig isoforms of its receptor molecule E-cadherin but not the murine or rat isoforms.

Purpose: The purpose of this study was to evaluate the hypothesis that EC strains are better adapted to infect human hosts, while strains harboring a PMSC in *inlA* are better adapted to (1) survive and grow under food-associated stresses and on foods or (2) infect other hosts where InlA-mediated invasion is not required.

Methods: A set of four EC and four *inlA* PMSC strains were characterized by (1) broth culture growth assays to simulate food-associated stresses (e.g., refrigeration, salt, and acidic pH), (2) Ready-to-Eat deli meat (formulated without antimicrobials) inoculation studies to evaluate growth on a food matrix, and (3) virulence assays in human and murine cell lines.

Results: Broth growth curves performed at 7°C to simulate refrigerated storage and food-associated stress conditions revealed that all strains reached similar final populations under multiple pH conditions (i.e., pH 7.4, 6.5, and 5.5) as well as in broth with the addition of 2.2% salt. All *inlA* PMSC strains reached the reached resultant population levels of > 6.3 log CFU/cm^2 turkey breast after 28 days of refrigerated storage, while EC strains showed > 2.0 log CFU/cm^2 variation in final populations (ranging from 4.4 to 6.6 log CFU/cm^2) at 28-d post-inoculation. Intracellular growth assays revealed that EC strains grew to higher levels by 9 h ($P = 0.0018$), but by 12 h no significant differences existed between the two groups

($P > 0.05$). Cytotoxicity assays demonstrated that *inlA* PMSC strains are more cytotoxic at 5 and 12 h post-infection in mouse macrophage cells ($P = 0.0311$ and $P = 0.0050$, respectively) than EC strains. Interestingly, *inlA* PMSC strains demonstrated increased invasion ($P = 0.0066$) in the L2 mouse cell line compared to EC strains.

Significance: These data suggest that epidemic clone strains may be better adapted to being pathogens in a human host, whereas strains harboring a PMSC in *inlA* may be better adapted to grow in foods and infect non-human hosts.

T8-08 Molecular Detection of *Listeria monocytogenes* in Small and Very Small Ready-to-Eat Meat Processing Plants DSC

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Introduction: Listeria monocytogenes is a foodborne pathogen that can cause listeriosis, a serious invasive disease in humans. This pathogen represents a significant challenge for the Ready-to-Eat (RTE) food industry due to its common isolation from virtually all environments along the food chain.

Purpose: The purpose of this study was to (1) use a combined molecular and cultural detection approach to track *L. monocytogenes* contamination patterns in six small or very small RTE meat processing plants in Colorado, Kansas, and Nebraska and (2) provide plant employees with basic knowledge about the nature of *L. monocytogenes* contamination and its control.

Methods: A total of 688 environmental sponge samples were collected from non-food contact surfaces in year 1 and a total of 896 samples were collected in year 2, including environmental sponge samples from non-food contact surfaces and food contact surfaces along with finished RTE meat products in some plants. Samples were collected from each of the six plants bi-monthly over a two year period. Samples were analyzed following a modified version of the US Food Safety and Inspection Service procedure for the *L. monocytogenes* BAX® screening test. In between year 1 and 2 of sample collection, an in-plant training session was conducted in each plant to provide all plant employees with information regarding the presence of *L. monocytogenes* in the plant environment and its control; results from the first year of testing were also presented to plant employees.

Results: Overall, *L. monocytogenes* was isolated from 6.1% of samples in year 1 and 4.6% of samples in year 2, where the prevalence of this pathogen ranged from 1.67% to 10.8% across different plants during year 1 and 0 to 8.21% across different plants during year 2. Statistical analysis showed that overall *L. monocytogenes* contamination was marginally reduced ($P = 0.1079$) in year 2 as compared to year 1. A single food contact surface and a single finished RTE meat product sample tested positive for *L. monocytogenes* in year 2. Comparison of pre- and post-training evaluations indicated that employees in all plants increased their knowledge of *L. monocytogenes* control.

Significance: Our findings highlight the importance of combined environmental testing for *L. monocytogenes* to track contamination patterns and in-plant training sessions to provide basic knowledge regarding the nature of *Listeria* contamination and its control in the plant environment.

T8-09 Biocide Use and Association with Antimicrobial Resistance of *Salmonella* Recovered in Swine Barn Floors

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Introduction: Swine are important reservoirs of *Salmonella*. The emergence and dissemination of multidrug resistant (MDR) *Salmonella enterica* has also become a major concern globally. Longitudinal studies on persistence of *Salmonella* before and after disinfection of swine barns with commonly used biocides in conventional commercial swine production systems are limited.

Purpose: This study was part of a larger project to determine the role of specific classes of biocides and heavy metal micronutrients in the emergence and persistence of multidrug-resistant *Salmonella* in swine production environments.

Methods: Barn floor swab samples were aseptically collected using conventional drag swabs before disinfection (n = 1,080) and after disinfection (n = 1,080) from randomly selected pens (n = 10 per barn in three replicates) in 36 barns. Samples were cultured for *Salmonella* isolation. Isolates recovered before and after disinfection were tested for antimicrobial resistance to a panel of 12 antimicrobials.

Results: A significant reduction of *Salmonella* was detected in post-disinfected barn floors (6.5%) as compared to pre-disinfection (15%). Of the total *Salmonella* isolates recovered from barn floors before disinfection (n = 447) and after disinfection (n = 310), 86.8% and 96.8% of the isolates were resistant to one or more of the antimicrobials tested respectively. A significant reduction in pansusceptible isolates was detected in isolates recovered from barn floors after disinfection (3.2%; 10/310) as compared to isolates recovered before disinfection (13.2%; 59/447) indicating the increase in resistant strains. A high proportion of multidrug-resistance (≥3 antimicrobials) was detected in *Salmonella* isolates after disinfection (65.8%) compared to those isolates recovered before disinfection (54.1%).

Significance: Even though a reduction of *Salmonella* was detected in swine barn floors after disinfection, *Salmonella* was still recovered from many of the barns. The increase in proportion of isolates with antimicrobial resistance in post-disinfection barns tends to be associated with specific classes of biocides, particularly the quaternary-ammonium glutaraldehyde combination (Synergize®). The highest increase in proportion of resistant isolates was to tetracycline, streptomycin, and sulfisoxazole, which might be associated with efflux systems induced by the biocides or persistence of class 1 integrons.

T8-10 Reduction in *Salmonella* Positives for Six USDA-FSIS Regulated Product Classes

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Introduction: In 2006 FSIS introduced a risk-based sampling initiative for PR/HACCP *Salmonella* sample scheduling. This initiative included the introduction of a risk-based algorithm to schedule *Salmonella* sampling, the categorization of establishments depending on the level of process control, as determined by *Salmonella* set results, and the introduction of PR/HACCP testing for turkey carcasses.

Purpose: The numbers of *Salmonella* positives and the proportion of human health-hazard serotypes (HHS, as identified by CDC) were compared for the first two years of risk-based sampling (July 2006 to June 2007 (year 1-risk based:Y1RB) and July 2007 to June 2008 (year 2-risk based:Y2RB)) and the preceding year (non-risk based sampling: NRB, July 2005 to June 2006, for six product classes: cow/bull, market hog, steer/heifer, ground beef, ground chicken and ground turkey. The effect of the season of sample collection and the progress toward the goal of 90% regulated establishments in category 1, i.e., demonstrating consistent process control by 2010, were also examined.

Methods: SAS software was used to extract, clean and analyze data retrieved from the FSIS Sybase data warehouse.

Results: Overall there was a decline in *Salmonella* percent positives for all product classes, with a significant decline in ground chicken, from 52.9 % in NRB to 23.5 % in Y2RB ($P < 0.05$). The overall proportion of HHS did not differ significantly for any product class from year to year. However, ground chicken and turkey had the highest proportions positive for HHS for three years. Furthermore there was no seasonal variation in *Salmonella* percent positive samples, although HHS were significantly more common in the winter ($P < 0.05$). In Y2RB, 70% of the establishments were in category 1, which was a 20% increase from NRB.

Significance: These data suggest that the risk-based sampling program had a positive impact, and the establishments are demonstrating progress toward consistent process control.

T8-11 Effect of Autoinducer-2 on the Gene Expression of *Salmonella* Typhimurium

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Introduction: Salmonella Typhimurium is the most common among the 2500 strains of *Salmonella*, the enteric pathogen that causes salmonellosis. This foodborne illness accounts for 1.3 million human illnesses due to food and 31% of food-related deaths in the United States each year. *S.* Typhimurium is known to use the signaling molecule called autoinducer-2 (AI-2) to communicate with other bacteria in response to external stimuli. In other bacteria, AI-2 is known to regulate various cellular processes such as biofilm formation, motility, and virulence. Understanding the role of AI-2 on the global gene expression profile of *S.* Typhimurium could pave the way for the use of AI-2 as a novel intervention tool.

Purpose: The purpose of this study was to understand the influence of autoinducer-2 on global gene expression of *S.* Typhimurium.

Methods: A poultry isolate, *S.* Typhimurium was obtained from the National Veterinary Service Laboratory, Ames, Iowa. A luxS mutant of this strain, designated PJ002 (luxS::cat), was generated. Fresh culture of PJ002 was inoculated in LB media (90%) + 10% of in vitro synthesized-AI-2 (test -1), LB media (90%) +10 % of spent culture media containing AI-2 from *S.* Typhimurium (test -2), LB media (90%) +10 % of spent culture media containing AI-2 from *E. coli* (test -3), and 10 % PBS + 90% of LB media (control) and cultured at 37°C until it reached OD 1.0. Total RNA was extracted from the samples and used for cDNA synthesis. The cDNA from control and tests was labeled with either cy3 or cy5 and hybridized on the *S.* Typhimurium oligo array. Arrays were subsequently analyzed, using Genepix pro 6.0 and SAS 9.1.3. Genes showing greater than 1.5 ($P < 0.05$) were identified as significantly differentially expressed genes.

Results: AI-2 appears to control the expression of a number of key metabolic genes and other genes controlling transcription, translation, and virulence. Genes including hilA, hilC, hilD, invF, orgC, sprB within the *Salmonella* Pathogenicity Island -1(SP-1), a Type III secretion system involved in bacterial colonization, were down-regulated in the presence of AI-2. These genes have been previously shown to be important for colonization in poultry and beef cattle. Infectivity assays also confirmed that AI-2 reduced invasion of HeLa cells.

Significance: The presence of AI-2 reduces the virulence of *S.* Typhimurium. Strategies to maintain high concentrations of AI-2 within the host may serve as an effective strategy to control colonization in the host.

T8-12 Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Pigs and Farm Workers

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Introduction: Methicillin resistant *Staphylococcus aureus* MRSA) has been identified from food animals, primarily pigs, in Europe and Canada. Currently, there is no information on the occurrence and magnitude of MRSA in swine herds reared in various production systems in the United States and its possible implications for occupational and public health.

Purpose: This study was undertaken to determine the prevalence of MRSA and other Methicillin-resistant staphylococci in swine at the early finishing stage and humans working in close proximity with pigs in Ohio.

Methods: We screened 120 pigs in five farms in Ohio for MRSA. Swab samples were collected from both anterior nares and fecal samples directly from the rectum of selected pigs of 6 to 9 weeks of age. At the same time, nasal and oropharyngeal swab samples were collected from consenting farm workers in close contact with pigs. Samples were processed following conventional cultural methods. Multiplex PCR was employed to detect mecA gene and staphylococcal chromosomal cassette (SCCmec) typing was done.

Results: None of the pigs were positive for MRSA. However, methicillin resistant coagulase negative Staphylococcus species (MRCoNS) were detected in 18% (22/120) of the pigs examined. MRSA was detected in one of the 17 farm workers included in this study. From the corresponding fecal samples collected from pigs, coagulase negative *Staphylococcus* species were detected in 1% (2 out of 120). The mecA gene was detected in coagulase negative *Staphylococcus* isolates using multiplex PCR. Staphylococcal chromosomal cassette (SCCmec) type I and IV were most commonly detected among the isolates.

Significance: While MRSA has not been identified from any of the farms in pigs, the high rate of MRCoNS may be a concern as these species have been reported as potential sources of the methicillin resistance gene to susceptible *S. aureus*. Identification of MRSA from a farm worker may also be a concern as farm workers could also act as potential sources of MRSA to pigs.