IAFP 2012 Abstracts

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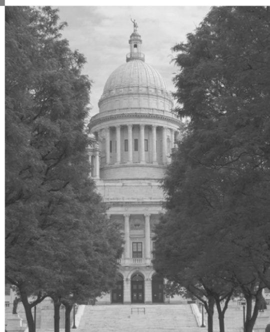

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Ivan Parkin Lecture

Industry and Government Roles in Food Safety Controls – A Perspective from Two Sides

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College Park, Maryland

Working for an industry trade association and for FDA has given me the opportunity to see how the industry and government carry out their roles in ensuring the safety of food in a complementary way. I also better see how we have become more proactive over the years and how we can work more cooperatively to enhance food safety.

Both industry and government must assess the risk of illness or injury from food. Industry generally assesses this qualitatively by identifying and evaluating the hazards associated with the food being produced. Industry uses information from a variety of sources, including government. Recently there has been more emphasis on doing quantitative microbial risk assessments. Industry rarely does a quantitative risk assessment, since this is not needed to determine appropriate control measures for a hazard, but industry is beginning to see how conducting such risk assessments can benefit them, e.g., to support labeling a product "pasteurized." Government is more likely to conduct quantitative risk assessments to describe the risk to consumers, which then become resources for industry in assessing hazards in, or risk from, specific food products. To conduct such risk assessments, the government must often rely on industry data. Although industry and government need data from each other to assess the risk from foods, both industry and government have issues related to data sharing, especially availability of data and timeliness.

Implementation of control measures is industry's role, but government regulations are often needed to establish the standards that industry as a whole must follow. In the absence of government regulations or guidance, industry must establish its own standards, but in the absence of regulations these may not be uniformly applied. Industry often recognizes a need and takes action well before the government can develop regulations and/or guidance (a slow process, even when government uses "expedited" approaches, because of the many layers of approvals needed). There are many examples in which industry has been proactive and moved much more quickly than government, including guidance on pathogens in refrigerated foods and low moisture foods. The government is willing to participate in the development of industry guidance documents, which can lead to a common understanding of the issues to be addressed and help ensure industry guidance will be acceptable to the relevant government agency. Sharing guidance documents with government can lead to disseminating them on a government website and to the development of agency guidance via a shortened process. Such sharing also has an advantage for industry in that government guidance is more likely to be practical for industry.

Recently much more emphasis has been placed on validating that control measures can achieve desired outcomes. Validation of control measures is primarily the role of industry, but government has much to offer in support of validation. Cooperative approaches can ensure acceptance of specific control measures by both industry and government.

The passage of the Food Safety Modernization Act is providing many opportunities for industry and government to work together to share food safety data and information, e.g., in the development of training and education materials and guidance documents. Industry and government should not waste these opportunities to work together to enhance the safety of the food supply.

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John H. Silliker Lecture

Challenges in Food Security and Food Protection

Dr. Catherine Woteki

Under Secretary for Research, Education, and Economics (REE)
and the Department's Chief Scientist
U.S. Department of Agriculture
Washington, D.C.

With global population expected to reach 9 billion by 2050, our agricultural systems are facing enormous challenges to produce enough food for all who will need it. Research and education are the best tools available to address that challenge, as sustainable intensification will be needed to boost agricultural production on a limited supply of arable land. To protect our natural resources and provide a nutritious diet for all, we will need to work on both securing enough food and ensuring that the food supply promotes life-long health. Those involved in nutrition and food safety understand that a food that is not safe is not nutritious. And we need to consider the supply chain from farm to table. Technologies can be helpful in reducing the estimated 40 percent of crops that are lost pre- and post-harvest to rodents and rot.

Scientists around the globe are making a priority of current research into crop diseases such as the wheat pathogen UG99, which threatens a key staple of many countries' diets. Protecting our food supply requires ongoing research into both plant and animal diseases that could, in a short period of time, be devastating. Just as new breeds of disease-resistant plants are developed, new diseases emerge that require new solutions. Both plant and animal specific as well as zoonotic pathogens are constantly evolving, and research is critical to staying ahead of the threat pathogens pose to crops, food animals and humans.

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Symposium Abstracts

Special Session Anatomy of Product Tracing on Sushi: Search for the Smoking Gun

DOUGLAS MARSHALL, Eurofins Scientific Inc., Fort Collins, CO, USA
THANE HANCOCK, Centers for Disease Control and Prevention, Atlanta, GA, USA
SHERRI MCGARRY, U.S. Food & Drug Administration, College Park, MD, USA
KARI IRVIN, U.S. Food and Drug Administration, College Park, MD, USA
DAVID NICHOLAS, New York State Department of Health, Troy, NY, USA
DOUG BRINSMADE, Sea-Delight, Miami, FL, USA

Between January 28 and May 1, 2012, 258 persons from 24 states and the District of Columbia were reported to be infected with *Salmonella* Bareilly or Nchanga, a strain which emerged later in the investigation. Collaborative investigation by state, local, and federal public health agencies indicated that a frozen raw yellowfin tuna product, Nakaochi Scrape from Moon Marine USA Corporation, was linked to the outbreak. Scrape is tuna backmeat that is scraped from the bones of tuna and may be used in sushi, sashimi, ceviche, and similar dishes. Compounding a rapid recall is that the suspected product may have passed through many distribution centers and was not clearly labeled. This recall came during the timeframe when FDA was conducting mock traceback/traceforward exercises on fresh tomatoes and a Kung Pao-style chicken product. If the outcomes from those trials had been available for application to the sushi outbreaks, would tracing the "smoking gun" have been more efficacious? This special session will hear from an epidemiologist, a major tuna supplier, and FDA on its work with CDC and future practices in product tracing.

S1 FSMA from Legislation to Implementation

JENNY SCOTT, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
PAMELA WILGER, Cargill, Inc., Wayzata, MN, USA
CARLOS ALVAREZ ANTOLINEZ, European Union, Washington, D.C., USA
PURNENDU VASAVADA, University of Wisconsin-River Falls, River Falls, WI, USA

With the enactment of the United States Food Safety Modernization Act in January 2011, there are new responsibilities for food companies, with an emphasis on comprehensive prevention controls in human and animal food facilities. The Act mandates prevention standards, strengths hazard analysis and risk-based preventive controls, prevention in imports, and provides produce safety and intentional adulteration standards. These extend to controls over imported food, new fees for food companies and importers and enhanced enforcement powers for U.S. regulatory agencies, such as the Food and Drug Administration. This symposium will provide IAFP attendees with current information on new policies and newly published guidelines that have been adopted or revised and enacted under the new law. With emphasis on food safety/preventive controls (HACCP), supply chain management, records maintenance and access and food defense plans. Integral parts of the Act cover prevention standards, inspection and compliance, imports, U.S. Federal/State integration and fees. A major focus of the Act also clarifies the need for third party certification, foreign supplier verification activities and training for food safety employees and supervisors. The speakers in this symposium will touch on these subjects after an overview of the Act and an update of the new regulations that have been put forth by the FDA is presented. Attendees will therefore gain an appreciation of FSMA affects on third party certification, foreign supplier verification and the implementation/training of food safety professionals. Finally, attendees will also gain an appreciation on the European perspective of FSMA and how the continent is responding to the requirements in the Act.

S2 Microbial Safety of Dry Spices

MARGARET HARDIN, IEH Laboratories & Consulting, Lake Forest Park, WA, USA
MICKEY PARISH, U.S. Food and Drug Administration, Washington, D.C., USA
NATHAN ANDERSON, U.S. Food and Drug Administration, Summit-Argo, IL, USA

A spice is defined as any aromatic vegetable substance in whole, broken or ground form whose function in food is primarily for seasoning, rather than for nutritional value, and from which no portion of any volatile oil or other flavoring principle has been removed. Spices and herbs are valued for their unique flavors, colors and aromas and are among the most widely used ingredients in food preparation and processing throughout the world. Unfortunately, there are multiple opportunities for microbiological contamination of spices during pre- and post-harvest operations. Contaminated spices have been responsible for outbreaks of salmonellosis and the presence of *Salmonella* spp. is of particular concern when herbs and spices are added to ready-to-eat foods. Spore-forming bacteria (*Bacillus cereus*, *Clostridium perfringens*), capable of causing foodborne disease when ingested in large numbers, are also frequently found in spices and herbs, typically at low levels. Because most spices are imported, this presents the additional challenge of verifying the safety of foreign suppliers. Spices are often treated with ethylene oxide, propylene oxide, or by gamma-irradiation. Nevertheless, fumigation of bulk packaged spices and herbs is difficult, and gases are not as effective on spores as on vegetative cells. Further, the toxicity of these fumigation methods has been called into question. While gamma-irradiation has the advantage of high-density penetration, it can also negatively affect sensory characteristics. Other decontamination methods that have been considered include steam treatment, dry heat treatment, irradiation by ultraviolet light, infrared or microwaves, high hydrostatic pressure, ozone, as well as treatment by fumigants such methyl bromide and sulfur dioxide. This symposium will address problems associated with various types of spices, the FSMA Foreign Supplier Validation Rule and means of mitigating microbiological risks associated with spices.

S3 Environmental Assessments (Root Cause Analysis) during Foodborne Disease Outbreaks

CAROL SELMAN, Centers for Disease Control and Prevention, Atlanta, GA, USA
SHERRI MCGARRY, U.S. Food & Drug Administration, College Park, MD, USA
LARRY KOHL, Food Lion Family - Delhaize America, Salisbury, NC, USA
PAT MALONEY, Brookline Health Department, Brookline, MA, USA
CATHY BUREAU, Buffalo Wild Wings, Minneapolis, MN, USA

Many investigations of foodborne disease outbreaks/food contamination events do not identify the contributing factors and environmental antecedents (root causes) for the outbreak. This symposium will describe actions being taken by the U.S. Food and Drug Administration,

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Symposium

the Centers for Disease control and Prevention, local environmental health staff and industry to ensure that environmental assessments are conducted in these situations and how the findings will be used.

S4 The Impact of Climate Change on Food Safety: Using Korea as an Example

KI-HWAN PARK, Chung-Ang University, Anseong, Gyeonggi, South Korea
SANG-DO HA, Chung-Ang University, Ansung-Si, South Korea
DEOG-HWAN OH, Kangwon National University, Chunchon, South Korea
HYANG SOOK CHUN, Korea Food Research Institute, Sungnam, South Korea

The symposium will focus on the issue of food safety and climate change in Korea. The Korean Food and Drug Administration has funded a 5-year project on climate change and food safety with KWR 12.5 million conducted by the Research Group on Food Safety Control Against Climate Change headed by Ki-Hwan Park at Chung-Ang University, Ansung. The goal is to develop green processing technology to take anticipative action for food safety against climate change and achieve low-carbon, green growth goal. This is to forecast the occurrence of foodborne disease caused by climate change and evaluating its influence on food safety. Steps will be taken to ensure the safety of agricultural, livestock and marine products in areas where temperatures have increased and the area is now subtropical. The presenters will outline various project areas such as: collecting and analyzing data on climate change and developing a predictive model; evaluating the influence of chemical, biological, and physical hazards due to climate change; introducing the hazard control process technology and low-carbon, green technology and improving consumer consciousness with regard to climate change and promoting international cooperation. A prediction and impact assessment on food safety due to climate change will be conducted an impact analysis of food risk factors through simulation of climate change and control management. Food safety control methods will be developed and establishment and utilization of a food risk communication system. A food crisis warning system and simulated training program will be developed.

S5 Today Their Problem – Tomorrow Ours: Impact of International Trade on Food Safety

MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA
PETER GERNER-SMIDT, Centers for Disease Control and Prevention, Atlanta, GA, USA
PETER BEN EMBAREK, World Health Organization Office, Geneva, Switzerland
DANIEL BAUSCH, Tulane University, New Orleans, LA, USA
RENE HENDRIKSEN, National Food Institute and Technical University of Denmark, Lyngby, Denmark
STEFANO MORABITO, Istituto Superiore Di Sanita, Roma, Italy

Background: A greater knowledge of different kinds of organisms based on region of origin is warranted based on increased volumes of international trade in foods. New pathogens, new food products and the growing volume of imported foods are an increasing challenge to food safety. What unusual types or sub-types of pathogens might be associated with various international regions? Are these pathogens associated with foods? Description: Import of a variety of food products and ingredients into the U.S. continues to increase. This symposium will explore unusual pathogens or pathogen subtypes (*Salmonella* serotypes, and *Escherichia coli*serotypes) which might be encountered in imported foods from different parts of the world (Asia, Australia, South America and Eastern Europe) and what unusual foodborne disease outbreaks occur in different regions.

S6 Control of Virus Contamination in Food Supply Chains

KRIS WILLEMS, University Leuven, Brussels, Belgium
NIGEL COOK, The Food and Environment Research Agency, York, United Kingdom
GARY RICHARDS, U.S. Department of Agriculture-ARS, Dover, DE, USA

The theme of this session is to describe methods for the control of virus contamination in the food chain. Topics will include the application of standardized methods for virus detection in food chain monitoring, advances in quantitative viral risk assessment, new paradigms in HACCP for foodborne viruses and the control of viruses in shellfish production.

S7 Measuring and Managing Norovirus Cross-contamination Risks in the Food Service Environment

CAROL SHIEH, U.S. Food and Drug Administration, Summit-Argo, IL, USA
STEPHEN GROVE, Institute for Food Safety and Health, Bedford Park, IL, USA
DONALD SCHAFFNER, Rutgers University, New Brunswick, NJ, USA
MIRIAM EISENBERG, EcoSure, a Division of Ecolab, Lincolnshire, IL, USA

Infection with norovirus (NoV) is considered the most common cause of foodborne illness in the U.S. Many foodborne NoV outbreaks result from consumption of food contaminated by an infected food handler in the foodservice environment. Such infections typically occur in closed environments including restaurants, cruise ships, schools and nursing homes where large amounts of food are prepared daily. Fresh fruits and vegetables have been implicated as vehicles for NoV. Preparation of fresh fruits and vegetables usually involves considerable human contact, including handling, chopping/slicing and mixing, and since these foods are often consumed raw, there is often no effective pathogen reduction step prior to consumption. Since 2008, researchers from the Institute for Food Safety and Health (formerly National Center for Food Safety and Technology), Rutgers University, and collaborators from FDA's Center for Food Safety and Applied Nutrition and Ecolab USA, have been collaborating to examine cross-contamination of NoV during common procedures used in preparation of fresh produce in a food service setting. The NoV transfer data, which has been collected using more than 120 volunteers, is being incorporated into a quantitative risk assessment to evaluate the risk reduction of worker behavior as a result of the research findings. The symposium will provide an opportunity for the project team to report their findings, discuss the potential for NoV transmission through a retail food service setting, as well as educational elements to change working behavior.

S8 Making a Difference: Data Collection for Risk Assessments through Innovative Approaches

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ROBERT BUCHANAN, University of Maryland, College Park, MD, USA

Constant challenges in developing microbial risk assessments are data quality and availability. Additional creative efforts are needed to address some of the data needs that often limit the potential of risk assessments to inform risk management decisions. This symposium will bring together speakers from government, academia and industry to share their experiences and lessons learned in generating data to support risk assessments. These include targeted data collection and leveraging novel data sources (e.g., conducting site visits to farms and manufacturing facilities, creative means of leveraging industry data, and greater synergy/coordination among government/academia/industry collaborations). These efforts not only enhance data acquisition, but also foster a greater degree of outreach and communication with stakeholders of the risk assessments, which can be used to inform government policy and risk management decisions.

Symposium

S9 Food Packaging Sustainability: Food Safety with Sustainable Packaging

ALLAN BAILEY, U.S. Food and Drug Administration, Baltimore, MD, USA
ROGER BONT, Cargill, Inc., Minneapolis, MN, USA
KAY COOKSEY, Clemson University, Clemson, SC, USA

Sustainability is no longer just a catchy word, but rather an opportunity for food companies to grow in a way that is profitable and meaningful for society and, ultimately, a new method of promoting and maintaining growth for the entire food industry. Reducing energy usage globally provides a sustainable medium to grow in, thereby maintaining a healthy balance of potential savings and consumption. With this in mind, we can improve the reusable social wealth of each region and expand the global resources that are accessible and will be readily available for future generations. The majority of our packaging materials are ending up in landfills. By using the several available options, the industry can reduce loading stresses on municipal solid waste streams. The collaboration of industry, consumers and regulatory agencies working together and applying all available techniques to minimize the adverse impact of packaging solid waste on the environment is very important to society. Sustainable packaging is a long term, consumer focused strategy which requires innovation in providing services to the consumers. A global, multi-dimensional and integrated approach is the key to the success of sustainable packaging. With material technology progress, new materials will extend the shelf-life of each product and expand market share. A new sustainable economy will be generated which will provide new business growth opportunities. This globally minded symposium will focus on current state-of-the-art developments and strategies relative to food packaging that is at once safe, sustainable and affordable.

S10 The Pre-harvest Conundrum: Efficacy Versus Adoption of Food Safety Interventions

TODD BRASHEARS, Texas Tech University, Lubbock, TX, USA
MORGAN SCOTT, Kansas State University, Manhattan, KS, USA
GUY LONERAGAN, Texas Tech University, Lubbock, TX, USA

Efficacious pre-harvest interventions are either available or will very soon enter the marketplace. Yet optimism surrounding these technological advances is somewhat tempered because none of these interventions are a 'silver bullet' even though their efficacy is consistent from study to study. The question then is, 'should we adopt these imperfectly efficacious interventions?' To help answer this, we might quantify the expected public-health benefits as impact is function of both efficacy and extent of adoption. In other words, broad adoption of a poorly efficacious product can improve public health whereas a hypothetical 'silver bullet' has no impact when left on the shelf. Consideration of adoption, therefore, is just as important as consideration of efficacy. Since adoption requires a behavioral change among producers (e.g., adoption of a new technology or best practice), we should explore not only the role of economics but also other critical determinants of behavior that include knowledge and a range of social norms and felt moral obligations. A quantitative understanding of these subjective and normative values facilitates construction of scenarios (or alternative operating realities) to test whether interventions are likely to be implemented or not. Ultimately, interventions must be perceived to be implementable by those responsible for that implementation and producers must also perceive that implementation is advantageous and results in some benefit. In this symposium, we will present information on efficacy, impact and challenges to adoption from an industry perspective then delve into avenues to effect behavior change through 'knowledge as in intervention' and systems thinking.

S11 *Salmonella* in Low-moisture Foods: A Continued Challenge

DON ZINK, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
SANGHYUP JEONG, Michigan State University, East Lansing, MI, USA
DONALD SCHAFFNER, Rutgers University, New Brunswick, NJ, USA
BRADLEY MARKS, Michigan State University, East Lansing, MI, USA
JOSEPH FRANK, University of Georgia, Athens, GA, USA
ELENA ENACHE, Grocery Manufacturers Association, Washington, D.C., USA

Human salmonellosis has remained a considerable challenge for the U.S. food industry, regulatory and public health agencies over the last decade. This challenge is illustrated by the fact that the annual incidence of human salmonellosis cases in the U.S. has not decreased over the last decade, despite the fact that the prevalence of a number of illnesses caused by a number of other foodborne pathogens has decreased considerably over the same time frame. For a variety of reasons, control of *Salmonella* in low-moisture foods represents a particular challenge. This symposium will include an introductory overview presentation on the challenges of controlling*Salmonella* in low-moisture foods as well as the outlook for improved and novel approaches to address this issue. Recognizing the challenges of controlling *Salmonella* in low-moisture foods, the ILSI North America Technical Committee on Food Microbiology has, over the last years, funded a number of research projects in this area. The rest of this symposium will focus in providing attendees with new information and science on*Salmonella* in low-moisture foods, resulting from these on-going research projects.

S12 What Goes around Comes around: Food Safety Concerns Associated with Water Re-use from Farm to Table

TREVOR SUSLOW, University of California-Davis, Davis, CA, USA
KEVAN MAIN, Mote Marine Laboratory, Sarasota, FL, USA
PHYLLIS POSY, Atlantium Technologies, Beit Shemesh, Israel

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SUZANNE TORTORELLI, Campbell Soup Company, Camden, NJ, USA
DAN BENA, PepsiCo, Vahalla, NY, USA

Sustainable water resource management has become a critically important issue for all segments of the global food supply chain. Conservation strategies focused on "re-use" may exhibit many forms, depending upon where and how water is used throughout the food production – harvesting – processing – packaging – distribution – consumption continuum. This symposium will address current/emerging practices, technologies, and concerns associated with water re-use, as well as provide practical, cost-effective strategies/solutions for ensuring the microbiological safety of re-use water in agriculture, aquaculture, and the food, beverage, and dairy manufacturing industries. Subject matter experts will address standards required for re-use water, and identify areas of opportunity for water technology companies to further develop and expand use of this important resource in food production and processing.

S13 HACCP – The Rise of the Prerequisites

SARA MORTIMORE, Land O'Lakes, Saint Paul, MN, USA
JOHN HOLAH, Campden BRI, Gloucestershire, United Kingdom
JANET SCOTT, PepsiCo Europe, Leicester, United Kingdom

Many *Salmonella* and *Listeria* outbreaks in recent years have been associated with cross-contamination of ready-to-eat (RTE) foods, after the cooking or decontamination step. Contamination in these foods is thus controlled by HACCP prerequisites, and may also be associated with the requirement for microbiological environmental sampling. RTE food manufactures are considering new prerequisite and environmental sampling models that are risk based and fulfill the requirements of U.S.- and EU-based legislation. This seminar will describe the work of Campden BRI and two global food manufactures in a new concept of prerequisite management. U.S. and EU factory studies have been initiated to determine the potential sources of microbial contamination (both harbourage sites and growth niches) and the vectors on which they could be transferred to food (air, solid and liquid contact). Once identified, controls for these sources and vectors are established and reviewed, such that potential contamination is reduced. Following HACCP principles of risk assessment to determine critical control points (CCP), the sources and vectors are risk assessed to determine those that are critical to the safety of the food, if controls should fail. Critical sources and vectors are described as Operational Prerequisites (OP) and control values, the validation, monitoring and verification of the controls, together with corrective actions and records etc. are established. Some of these controls and control values may involve the requirement for microbiological sampling, the results of which can directly be related to the risk of product contamination. Theoretical and practical approaches to this innovative risk assessment of operational prerequisites, that is applicable in the U.S. and Europe, will be presented.

S14 Recall Management and Best Practices

ROBERT WAITE, FoodTrack, Inc., Wellington, FL, USA
KATHY GOMBAS, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
BRIAN LYNCH, Grocery Manufacturers Association, Washington, D.C., USA

Consumer confidence in the food supply has changed dramatically over the last five years. In May 2011, the Food Marketing Institute announced consumer confidence in food safety is at its highest point in seven years, with 88 percent of shoppers completely or somewhat confident in the safety of food at the supermarket. Recall management plays a significant role in maintaining this level of consumer confidence. Over the last five years there were several high profile recalls including pet food, fresh leafy greens and eggs. Following each recall event, consumer confidence declined. Recalls continue to increase every year, and they are becoming more complicated with ingredients contributing to ever expanding recalls (i.e., peanuts and hydrolyzed vegetable protein). It is essential for the food industry to partner with one another to implement innovative, best practices for proper execution of recalls at retail and food service. Not only will proper recall management improve consumer confidence, but it will also reduce and/or prevent expanded foodborne illness.

S15 Food Safety and International Trade: Opportunities and Challenges

ISABEL WALLS, U.S. Department of Agriculture-NIFA, Washington, D.C., USA
JEAN BUSBY, U.S. Department of Agriculture-ERS, Washington, D.C., USA
IAN JENSON, Meat & Livestock Australia, North Sydney, Australia
MIEKE UYTTENDAELE, Ghent University, Ghent, Belgium
DORY BARNINKA, JBS, Sao Paulo, Brazil
NATALIE DYENSON, Walmart, Fayetteville, AR, USA

Increased international food trade has led to new food safety challenges which have impacted movement of food products between countries. Availability of a variety of foods throughout the year and at lower prices has greatly benefited consumers worldwide. However, with food trade, the possibility of emerging and re-emerging food safety hazards and widespread contamination of food among various countries exists. Globalization of the food supply has raised the needs for: 1) defining the appropriate level of protection for foods from food safety hazards and using this level as a scientific basis for setting food safety standards, 2) using risk assessment at the international levels to evaluate the standards, and 3) providing food safety capacity building to ensure that the standards are met. This can help to open export markets and facilitate international trade. Setting food safety standards should result in food producers providing safer food to domestic and international markets; failure to do so will result in a negative public health impact and a wide range of business and economic losses. Our goal in this symposium is to discuss food safety challenges in relation to potential international trade barriers of food products. IAFP and non-IAFP members will address and offer potential solutions to the following areas: 1) lack of data for food safety risk assessments, 2) lack of resources to collect data particularly in developing countries, 3) lack of internationally accepted laboratory methods for quantifying/detecting hazards in imported/exported foods, and 4) how to improve collaboration/communication among international communities when making science-based decisions. Case examples will be included.

S16 Drug Residues in Milk and Milk Products Risk Assessment

JANE VAN DOREN, U.S. Food and Drug Administration-CFSAN-OFDCER, College Park, MD, USA
STEFANO LUCCIOLI, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
ROGER HOOI, Morningstar Foods/Dean Foods, Dallas, TX, USA
JOHN SHEEHAN, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
DAVID PLUNKETT, CSPI, Washington, D.C., USA
WENDY FANASELLE, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

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A variety of antibiotics and other drugs are approved for use in farm animals, including dairy cattle. There are concerns over the possibility of drug residues in dairy foods, such as fluid milk. At present in the United States, the current requirement is to test every bulk milk pick-up tanker before it is received in a milk plant. However, the testing for drug residues is limited to certain drugs of the Beta lactam antibiotic class only. The National Conference on Interstate Milk Shipments (NCIMS) Drug Residue Committee has requested that FDA perform a risk assessment on potential drug residues in milk and milk products produced in the United States to re-evaluate the drugs that FDA should require testing for in the bulk milk. This symposium will describe the potential drug residue concerns in milk and milk products and the approach FDA is utilizing in analyzing this issue. The Drug Residues in Milk Risk Assessment Work Group consists of scientific and dairy experts from FDA, Center for Food Safety and Applied Nutrition (CFSAN) and Center for Veterinary Medicine (CVM) working together to develop a risk assessment on drug residues in raw milk and milk products.

S17 Toxoplasma: Detection and Risks Associated with Other Diseases and Latent Infection – Prevalence, Methods, Detection in Meat and Poultry, and Burden of Foodborne Illness

DOLORES HILL, U.S. Department of Agriculture-ARS, Beltsville, MD, USA
MARIEKE OPSTEEGH, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
ROBERT YOLKEN, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

It is estimated that in the United States, domestically acquired foodborne *Toxoplasma gondii* causes over 86,000 illnesses; 4,400 hospitalizations and 320 deaths. Of the 31 major pathogens, *T. gondii* is the fourth (8%) leading cause of hospitalizations and second (24%) leading cause of deaths (Scallan, 2011). The University of Florida Emerging Pathogens Institute reported *T. gondii* is the second most burdensome foodborne pathogen (Batz, 2011). Batz (2011) calculated the combined "burdensome-rank" using quality adjusted life-year loss, cost of illness, and the illness/hospitalizations/deaths by Scallan (2011). Given these facts doesn't it seem strange that so many people don't really know about this protozoan parasite or the ways in which the disease may manifest? Scientists from The Netherlands, Brazil and the United States will review the prevalence of, and detection methods for, Toxoplasma in livestock (and meat products) in their respective countries/continents. An industry representative will discuss the efforts in the United States to reduce Toxoplasma in swine. One public health scientist will review the classic symptoms of *T. gondii* infections: congenital toxoplasmosis, ocular disease and disease in immunocompromised patients. Additionally this symposium will review the research showing that latent infections of *T. gondii* are linked to a wide range of diseases/syndromes/symptoms: human psychoses (including schizophrenia); delayed reaction time; and, traffic accidents.

S18 Microbial Transfer within Food Manufacturing Plants and Hygienic Zoning Control Verified by Environmental Monitoring

JOHN HOLAH, Campden BRI, Gloucestershire, United Kingdom
FREDERICK COOK, Malt-O-Meal Company, Lakeville, MN, USA
JENNY SCOTT, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
DONALD SCHAFFNER, Rutgers University, New Brunswick, NJ, USA
KRISTINA BARLOW, U.S. Department of Agriculture-FSIS, Fairfax, VA, USA
STEVEN TSUYUKI, Maple Leaf Foods, Toronto, ON, Canada

This symposium will address hygienic zoning for food plants and the barriers between zones that minimize potential for transfer of pathogenic microorganisms from potentially contaminated areas to finished food products. This food safety system may include multiple barriers incorporating physical and procedural controls, the designs of which depend on the type of product, layout of product flow within the plant and microbial reduction steps during product manufacture. This symposium will be valuable for those who work with environmental pathogen control in food plants such as industry quality assurance personnel, consultants, academicians and regulators. Also students will benefit from an understanding of these controls. This symposium is very timely especially for those who are working currently to implement enhanced environmental control. The first two speakers will present information on regulatory experiences with food contamination/illness incidents attributed to transfer of bacteria within plants, their up-to-date findings regarding location of pathogens in food plants with possible product contamination paths and examples of risky situations they have encountered that could be mitigated by enhanced hygienic zoning controls. The second two speakers will present examples of hygienic zoning control systems contributing to safety of high moisture (processed meat) products and low moisture (cereal) products. This will include specific descriptions of controls that prevent transfer of contamination by personnel, equipment and materials and their findings regarding effectiveness of controls. The fifth and sixth speakers will present information on studies related to understanding modes of contamination transfer such as hands, clothing, footwear, equipment, water and air.

S19 Foodborne Disease Outbreak Update

SHERRI MCGARRY, U.S. Food & Drug Administration, College Park, MD, USA
BENJAMIN SILK, Centers for Disease Control and Prevention, Atlanta, GA, USA
DALE MORSE, Centers for Disease Control and Prevention, Atlanta, GA, USA
KARI IRVIN, U.S. Food and Drug Administration, College Park, MD, USA
PATRICIA WHITE, U.S. Department of Agriculture-FSIS, Omaha, NE, USA
STEPHANIE DEFIBAUGH-CHAVEZ, U.S. Food and Drug Administration-CFSAN, Washington, D.C., USA

The symposium will provide lectures on the epidemiological and environmental investigations into the 2011 *Listeria monocytogenes* outbreak linked to fresh cantaloupe and the 2011 MDR *Salmonella* Heidelberg ground turkey outbreak. The symposium will also provide an update on what the U.S. Centers for Disease Control and Prevention and the U.S. Food and Drug Administration are doing to improve foodborne disease surveillance and response.

S20 Food Allergen Labeling: Challenges and Best Practices

JOSEPH BAUMERT, University of Nebraska-Lincoln, Lincoln, NE, USA
REIKO ADACHI, National Institute of Health Sciences, Tokyo, Japan
STEVEN GENDEL, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
THOMAS WIESTER, Campbell Soup Company, Camden, NJ, USA

More than 11 million Americans suffer from food allergies and young children are disproportionally more affected. Strict avoidance of the offending food remains the only effective means to prevent the occurrence of allergic reactions. Allergic consumers rely on food labels to

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disclose the presence of allergenic ingredients. Many countries have enacted food allergen labeling regulations. Globalization in food production, manufacturing and trade has made compliance with different requirements an ever-expanding challenge. Recent data have suggested that labeling errors are the leading cause of food allergen recalls. There is a critical, ongoing need for the food industry to develop and implement best practices to ensure accurate and proper allergen labeling. This symposium will provide IAFP attendees with an overview of the food allergen labeling requirements set up by various countries and how industry is working towards meeting these requirements. Issues or obstacles that contribute to errors in food allergen labeling will be discussed. Expert speakers will also showcase best practices to enhance compliance of food allergens regulations from a manufacturing, supply chain and a global trade perspective.

S21 Freedom Has a Thousand Charms: Gluten-free and How to Achieve It

MOHAMMED OBANNI, Hain Celestial Group, Modesto, CA, USA
THOMAS GRACE, Bia Diagnostics, Burlington, VT, USA
JOSEPH BAUMERT, University of Nebraska-Lincoln, Lincoln, NE, USA

The market for gluten-free food products has grown considerably over recent recent months, to satisfy the needs of actual gluten-sensitive consumers and others who perceive benefit in a gluten-free diet. In light of pending regulations regarding the manufacture and marketing of gluten-free products, this symposium will provide timely and valuable information on what is needed to achieve, prove and maintain gluten-free status. Experts in the field will discuss the background to scientific and regulatory issues, current industry best practices, testing requirements and certification schemes.

S22 China: Food Safety in an Emerging Market Economy

LEON GORRIS, Unilever, Shanghai, China
LESLIE BOURQUIN, Michigan State University, East Lansing, MI, USA
PAMELA WILGER, Cargill, Inc., Wayzata, MN, USA

A globally traded food supply has placed increasing concern on food safety within the People's Republic of China especially regarding the falsification of food products to increase the price and value of food. This symposia is designed to focus on food safety in China at the government, academic, and manufacturing levels. The academic presentation will discuss the role that Michigan State University has been involved with bridging food safety knowledge between the East and West. The concluding two presentations – examples of management systems at the manufacturing level, Unilever and Cargill for the approaches on product safety and sustainability. Collectively this symposium is meant to provide an integrated approach to bridging the gaps and understanding the current approaches for food safety within China.

S23 The Food Safety Modernization Act: Implementing the Provisions on Imported Foods

MARK ZINER, Department of Homeland Security, Washington, D.C., USA
KATHY GOMBAS, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

In 2011, the Food Safety Modernization Act was signed into law by President Obama. The law is a comprehensive overhaul of FDA's legal framework for the regulation of food. This session will review the progress FDA has made on implementing the provisions of the Act addressing the safety of imports. Currently between 10 and 15 percent of food consumed in the U.S. is imported, including many products regulated by the Food and Drug Administration. Nearly two-thirds of fruits and vegetables and 80 percent of seafood consumed domestically come from outside the country. Under the Act, FDA will implement a new Foreign Supplier Verification Program and develop new programs for working with foreign governments. This mini-symposium will provide a review of the elements of the new law as it governs food imports and provide an update from key stakeholders on the proposals for implementation.

S24 The Food Safety Modernization Act: Implementing the Preventive Controls and Other Aspects on Domestic Foods

RITA JOHNSON, Florida Department of Agriculture and Consumer Services, Tallahassee, FL, USA
JENNY SCOTT, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
JENNIFER MCENTIRE, Leavitt Partners, Frederick, MD, USA

In 2011, the Food Safety Modernization Act was signed into law by President Obama. The law is a comprehensive overhaul of FDA's legal framework for the regulation of food. This session will review FDA's approach to implementing the provisions of the Act addressing domestic foods. FDA is developing regulations on preventive control plans for all food processors and science based minimum standards for the safe production and harvesting of fruits and vegetables, following a number of serious outbreaks linked to domestic foods, including fresh vegetables and fruit. The law also contains requirements regarding traceability. This mini-symposium will provide a review of the framework for implementing the new law and an update from FDA and key stakeholders on the proposals for implementation of the process control and produce safety provisions, as well as progress on the traceability pilot projects.

S25 Local Foods: Food Safety Risks and Benefits

CATHERINE CUTTER, The Pennsylvania State University, State College, PA, USA
JAMES GORNY, U.S. Food and Drug Administration-CFSAN, Fulton, MD, USA
MICHELLE GREGG, Ohio Ecological Food and Farm Association, Columbus, OH, USA

There is considerable interest among some retailers and consumers to purchase foods grown locally. The stimulus driving this movement may be rooted in philosophical, environmental, economical or political systems of belief. Notwithstanding, the enhancements to quality and safety inherent to locally sourced foods is a debatable topic. The purpose of this interdisciplinary symposium is to explore multiple factors influencing the trend to local food preferences and discuss and highlight the impact that factors involved in the production and marketing of these foods may have on food safety. It is expected that this symposium will be of general interest to a wide spectrum of food safety professionals, including government, academic and industry IAFP members. Likewise, the topic addressed is relevant to a variety of food commodities including dairy, eggs, meat and poultry and fruits and vegetables. Speakers will address issues ranging from food safety knowledge and practices among small-scale producers who sell locally, the microbiological quality of foods available for at local markets, economics of locally sourced foods, consumers' food safety concerns/beliefs about local foods and regulations governing locally produced foods.

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S27 Food Defense: Where are We and Where Do We Have to Go?

SHAUN KENNEDY, University of Minnesota, Saint Paul, MN, USA
GALE PRINCE, Sage Food Safety Consultants, Cincinnati, OH, USA
BILL RAMSEY, McCormick & Co., Sparks, MD, USA
DANE BERNARD, Keystone Foods L.L.C., West Conshohocken, PA, USA
TED ELKIN, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
RYAN NEWKIRK, U.S. Department of Agriculture-FSIS, Washington, D.C., USA

At the IAFP 2011 Annual Meeting, the food defense symposia showcased a combination of innovative tools and programs available for industry and regulatory agencies worldwide to evaluate their knowledge and understanding, as well as improve their readiness for a possible attack on our food system. With that foundation, gaining an understanding of the level of readiness in the international community will help us benchmark our own preparedness and establish continuous improvement programs to address food defense. With the passage of the FDA Food Safety Modernization Act (FSMA) requiring the development of science-based regulations to protect against the intentional adulteration of domestic and imported foods, and the continued implementation of the USDA FSIS food defense plans, additional regulatory requirements are on the horizon. The additional FSMA requirements for managing the safety of imported foods, can create a complex food defense landscape to navigate. Economic adulteration continues to play a role in our food defense thinking. This symposium will provide stakeholders an update of the regulatory perspective on food defense from the FDA and USDA, a review of data driven signal detection systems, consumer attitudes towards agroterrorism, and observational perspectives of the current state of preparedness of the industry world-wide, to help us map for the future.

S28 Long Term Health Outcomes (LTHO) of Foodborne Illnesses and Their Contribution to Risk Assessment and Policy Evaluation

ARIE HAVELAAR, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
BARBARA KOWALCYK, Center for Foodborne Illness, Grove City, PA, USA
TANYA ROBERTS, Center for Foodborne Illness Research & Prevention, Vashon, WA, USA
ROBERT BUCHANAN, University of Maryland, College Park, MD, USA
KATE THOMAS, Public Health Agency of Canada, Guelph, ON, Canada
ALESSANDRO CASSINI, European Centre for Disease Prevention and Control, Stockholm, Sweden

The extent to which long-term health outcomes (LTHO) are incorporated into risk assessments and public policy decision making is an area for development. Acute impacts are widely appraised while long-term sequelae such as reactive arthritis, kidney disease, mental impairment and paralysis are poorly identified and quantified, both necessary steps for incorporating LTHOs into hazard characterizations in risk assessments. Evidence of the disease burden for LTHOs indicates that their importance can be as great as the acute burden for some pathogens. By ranking foodborne pathogens based on disease burden (both acute and LTHO) rather than incidence provides insights into the true societal and public health costs, and will help researchers and policy-makers identify major opportunities for prevention, control and the reduction of the disease burden. This symposium brings new research findings from Europe and North America to update our knowledge of the array and importance of LTHOs associated with foodborne illness and how public policies may influenced to improve food safety performance.

S29 STEC in Food: It's Time for Action!

JOSEPH BOSILEVAC, U.S. Department of Agriculture-ARS, Clay Center, NE, USA
IAN JENSON, Meat & Livestock Australia, North Sydney, Australia
JOHN RUBY, JBS, Green Bay, WI, USA
KERRI HARRIS, Texas A&M University, College Station, TX, USA
GUY LONERAGAN, Texas Tech University, Lubbock, TX, USA

Non-O157 STECs are now considered a top priority foodborne pathogen since they have been involved in various outbreaks linked to different food matrices around the globe (*Escherichia coli* O26 in ground beef and in cheese, *E. coli* O145 in lettuce, *E. coli* O103 in mutton sausages, etc.). With improved illness diagnostic procedures, they have also appeared in the CDC foodborne illness reports, and have been shown as representing a significant percentage of illnesses linked to hemolytic uremic syndrome and hemorrhagic colitis. In the U.S., the USDA-FSIS has now developed an analytical method for the screening of the six serogroups (MLG 5B) most frequently involved in clinical cases in humans (*E. coli* O26, O45, O103, O111, O121 and O145, also called "Big Six"). Simultaneously, commercial methods, mainly based on the real-time PCR technique have been developed and are now available to the food industry for routine testing of the Top six STECs. In September 2011, USDA-FSIS initiated the rule-making process declaring the Top six STECs as "adulterants of non-intact raw beef products and product components within the meaning of the Federal Meat Inspection Act (FMIA)". USDA-FSIS intends to implement this new regulation by March 5th, 2012. This new regulation will represent a significant challenge for the meat industry as well as the overall food industry. The meat industry has several concerns with this regulation, among which the performances of the current analytical methods, the percentage of "true positive meat samples", the implementation of the STEC hazard in their HACCP and verification plans, the prevalence of STEC among meat coming from various countries (Meat exporters to U.S. are also very concerned by this new regulation), and the preventive actions currently available for a better management of STEC risk. This symposium presents some of the latest data on rapid methods for STEC analysis, on STEC prevalence in bovine meat, on pre-harvest intervention technologies, on HACCP and implementation of a risk management of STECs in beef operations. The symposium will offer a tribune to the audience for discussion with the presenters.

S30 Drivers for Global Food Safety: Aligning Public, Private, and Government Resources

PATRICK WALL, University College Dublin, Belfield, Ireland
JEAN KAMANZI, The World Bank, Washington, D.C., USA
MARY SCHMIDL, IUFoST, Minneapolis, MN, USA
MICHAEL ROBACH, Cargill, Minneapolis, MN, USA
PETER BEN EMBAREK, World Health Organization Office, Geneva, Switzerland
KATHY GOMBAS, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

The key objective of this symposium is to bring together experts within the public, private and government sectors to discuss cooperation on the global level to ensure a safe global food supply chain. Within the holistic concept of an international umbrella of food safety, how well

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integrated are these partners in identifying and addressing the major challenges? EFSA, WHO/FAO, World Bank, IUFoST, FDA and Cargill representatives with international responsibilities for food safety will discuss how their individual organization drives a global safe food supply and how we can all help in this challenge. The goal is to learn from each other and engage in a more effective collaboration and cooperation with all parties to ensure a well-formulated, science-based approach to improve food safety and public health across the globe.

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S31 Harmonization of Methods to Evaluate and Validate Preventative Controls

LARRY KEENER, International Product Safety Consultants, Seattle, WA, USA
JOHN LARKIN, U.S. Food and Drug Administration-CFSAN, Bedford Park, IL, USA
RUSSELL FLOWERS, Silliker Group Corp., Chicago, IL, USA

The 111th U.S. Congress passed S510 in 2010 known as FDA Food Safety Modernization Act (FSMA), enacted Jan 4, 2011. There are now new responsibilities for food manufacturers and food producers. One of these responsibilities is to identify hazard and develop preventative controls based on that hazard and verification process that the developed preventative control significantly minimizes or prevents those hazards. In this whole new world of process control or development of a process to address a specific hazard, new questions arises on what and when to do and how to get to a specific goal. This symposium aims to address questions that food manufacturers and food producers will face and how academia and researchers can interact to address questions and issues on validation of processes.

S32 Improving Retail Food Safety: Studies on the Presence and Transmission of *Listeria monocytogenes* and Predicted Public Health Benefits of Changes in Retail Practices

JOHN LUCHANSKY, U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA
MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA
REGIS POUILLOT, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

Infections with *Listeria monocytogenes* cause an estimated 1,500 hospitalizations and 260 deaths in the United States each year, mostly attributable to consumption of contaminated ready-to-eat (RTE) foods. Cross-contamination of RTE foods during slicing or handling at retail groceries has been identified as a major risk factor. Many questions about how such foods become contaminated remain. Several federal entities (USDA and HHS), state health departments and academia have partnered to collect targeted data and conduct studies to address these questions. This symposium will explore the latest data on the presence, distribution, and transmission of *L. monocytogenes* at retail, as well as on food-handling practices at retail. In addition to this discussion, we will present a new interagency retail *L. monocytogenes* risk assessment model, based on these data, that addresses the retail behavior and practices that may contribute to or mitigate the risk of listeriosis from RTE foods. The session will conclude with an industry perspective on how to control *L. monocytogenes* cross-contamination at retail.

S33 Tales from the Food Safety World: A Collection of Extraordinary Stories from Our Profession

JEANNINE RIESS, Colorado State University, Fort Collins, CO, USA
SCOTT RUSSELL, University of Georgia, Athens, GA, USA
BENJAMIN CHAPMAN, North Carolina State University, Raleigh, NC, USA
PAUL HALL, AIV Microbiology & Food Safety Consultants, Inc., Overland Park, KS, USA
MARK CARTER, QC Laboratories, Southampton, PA, USA
MINDY BRASHEARS, Texas Tech University, Lubbock, TX, USA

This symposium will showcase a collection of interesting and exciting stories from the food safety world. It will be an interactive symposium through the use of an audience response system that will help to encourage audience participation. Attendees of this symposium will be greeted with a fun and lighthearted atmosphere; however, it will not be short on learning opportunities as topics will be presented by an expert in his/her food safety field, and will provide an informative and educational insight into a different area of food safety. A variety of topics were chosen to engage the audience, including sometimes overlooked topics (i.e., food safety at the local level from a public health inspector's perspective), topics of current focus (i.e., insights from both social media outlets and international food safety research projects), and topics that are popular and recurring at IAFP (i.e., investigative case studies from both an industry and academic perspective). Additionally, topics will be presented in a manner that may "gross" attendees out, will provide multiple opportunities for "I didn't know that!" moments, and will encourage audience participation, all leading to a very engaging symposium.

S34 Sprout Safety: What We've Done, What We've Learned and How We Can Continue to Move Forward

MICHELLE SMITH, U.S. Food and Drug Administration, College Park, MD, USA
TONG-JEN FU, U.S. Food and Drug Administration, Bedford Park, IL, USA
ROBERT SANDERSON, Jonathan Sprouts Inc., Marion, MA, USA
BOB RUST, International Specialty Supply, Cookeville, TN, USA
MANSOUR SAMADPOUR, LifeForce Foods, Lake Forest Park, WA, USA

Since 1996, contaminated sprouts have been linked to at least 30 outbreaks and over 2000 cases of illness in the U.S. Globally, the concern about consumption of raw sprouts has increased significantly after the 2011 outbreaks associated with contaminated fenugreek sprouts which resulted in more than 4000 illnesses and 50 deaths in Germany and France. Seed is often the source of contamination, but sprouts pose a particular concern as the conditions that promote germination of the seed also promote the growth of pathogens, if present. In 1999, FDA issued two guidance documents to industry recommending good practices that seed suppliers, distributors and sprout growers should undertake to address common risk factors in their operations. Although full implementation of the guidance is still a challenge, many members of the sprout industry have developed innovative approaches to minimize the risks associated with raw sprouts. This symposium will review the issues and challenges associated with sprout food safety from both an industry and a regulatory perspective. To ensure sprout safety, it is clear that collaboration among stakeholders across the "farm-to-table" continuum is critical. The symposium will also showcase a number of initiatives taken by the industry, government, and academia to help advance sprout food safety.

S35 Human Pathogens on/in Plants: Multidisciplinary Synergies for Enhancing Food Safety

MICHAEL MAHOVIC, U.S. Food and Drug Administration, College Park, MD, USA
JERI BARAK, University of Wisconsin-Madison, Madison, WI, USA
STEVEN RIDEOUT, Virginia Tech, Painter, VA, USA

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Recent produce-associated outbreaks of human diseases have demonstrated that foodborne human pathogens can be associated with plant products and crop plants can be contaminated in the field. *Salmonella enterica* in/on plants shows preferential bacterial colonization in the rhizoplane, bacterial growth on root surfaces and extended survival in soil when crops are planted subsequently. Most efforts have focused on potential postharvest solutions rather than addressing microbial-plant interactions in the field. Effective risk reduction and prevention strategies require knowledge of the interactions of foodborne pathogens with one another, with plants and with nonpathogenic microflora. Plant pathologists have extensive expertise on the complex relationships between microbes and plants and can be a valuable scientific resource to enhance fundamental knowledge of, and design effective solutions to, microbial contamination of food plants. Effective solutions will require the application of emerging research tools and strategies as well as creative cross-disciplinary research efforts. This symposium will provide examples of applying plant pathology principles and knowledge to solving human pathogen on plant issues, highlights of multidisciplinary research reported at the Human Pathogens on Plants Workshop: Multidisciplinary Strategies for Research (February 2012), FDA's strategic research agenda to support produce regulations and the role of extension in implementing safe production practices. The symposium is unique in that it focuses on the increasing need for multidisciplinary solutions to food safety – from plant pathologists and food technologies to extension professionals and regulators.

S36 Microbiological Safety of Fresh Produce

JAMES GORNY, U.S. Food and Drug Administration-CFSAN, Fulton, MD, USA
TREVOR SUSLOW, University of California-Davis, Davis, CA, USA
WILL DANIELS, Earthbound Farm, San Juan Bautista, CA, USA
ROBERT BRACKETT, Institute for Food Safety and Health, Bedford Park, IL, USA
ELIZABETH BIHN, Cornell University, Ithaca, NY, USA

The safety of fresh produce has been questioned during the past two decades based on increasing numbers of foodborne illness outbreaks. The importance of produce safety was punctuated in 2011 by historic outbreaks associated with fresh sprouts and cantaloupe, resulting in 1,000 laboratory-confirmed cases of illness and more than 70 deaths. Beyond problems associated with foodborne illnesses, each outbreak traced to fresh fruits and vegetables results in significant profit losses to the industry. Research studies have attempted to mitigate these problems by examining the behavior of foodborne pathogens within leafy greens and other fresh produce, identifying/eliminating point source contamination, and by determining the efficacy of decontamination treatments. These mitigations have been considered in the development and application of good agricultural practices and best management practices (GAPs and BMPs). The Food Safety Modernization Act (FSMA) will tie many of these areas together; impacting the way fresh produce is grown, processed and handled. This symposium will provide an overview of current issues regarding the microbiological safety of fresh produce.

S37 *Salmonella* in Shell Eggs – Post-harvest Intervention Technologies

GREGORY FLEISCHMAN, U.S. Food and Drug Administration , Bedford Park, IL, USA
DAVID GEVEKE, U.S. Department of Agriculture-ARS, Wyndmoor, PA, USA
AHMED YOUSEF, The Ohio State University, Columbus, OH, USA
JEAN JENSEN, Purdue University, West Lafayette, IN, USA

A potent source of *Salmonella* Enteritidis (SE) is shell eggs; so potent that the Food and Drug Administration promulgated the extensive Egg Rule (21CFR16, 21CFR118) to reduce the incidence of SE at the egg production level. This rule has 13 provisions, with a producer being exempt from all but two minor provisions if shell eggs are further processed to receive a 5 log reduction. Indeed, it has been estimated that if all shell eggs in the U.S. received a 5 log pasteurization and were stored at 7.2°C within 0.5 days, yearly illnesses would be reduced from 130,000 to 2,700. The existing pasteurization approach of hot water immersion handles a very small number of the roughly 65 billion eggs produced annually in the U.S. and creates only a niche market. Large scale cost-effective pasteurization is clearly needed. In this symposium, experts in their fields will discuss the challenges of dealing with SE in shell eggs. This will include the most recent information on current shell egg pasteurization methods and promising novel intervention technologies including gaseous ozone, microwave heating and rapid cooling. Attendees will come away with an increased knowledge of the actual threat that SE poses, the current state of SE prevention in shell eggs and the anticipated approaches to eliminating it.

S38 Sanitation Challenges in the Retail Food Kitchen

HALEY OLIVER, Purdue University, West Lafayette, IN, USA
GINA NICHOLSON, The Kroger Company, Cincinnati, OH, USA
JEFFREY ANDERSON, Procter and Gamble Professional, Covington, KY, USA

The retail food kitchen has become a complex food processing room. However, most retail food kitchens are not closed processing environments like most food manufacturing plants. The open format makes it difficult to reduce unintended contamination. Retail kitchens see many visitors from delivery people, maintenance technicians, school tours and even senior managers and executives. Recent research conducted by Purdue University and Cornell University reveals that the standard sanitation operating procedures commonly used by retail food businesses do not accurately clean food contact surfaces, touch points of non food contact surfaces or floors walls and drains. Development of validation of cleaning and sanitation processes at retail around hot and cold processing equipment in delis and prepared foods venues is needed. Many retailers have been researching affordable methods of validation that a low literacy workforce can use successfully. This symposium is designed to share the current research on proper validation systems for cleaning retail food kitchens and how to train and coach food employees on these new systems.

S39 Translating HACCP to Lean, Six Sigma – Learning How Food Safety Fits into the Process Improvement Model

LARRY KOHL, Food Lion Family - Delhaize America, Salisbury, NC, USA
KEN DAVENPORT, 3M Microbiology, Saint Paul, MN, USA

Food Safety and Quality professionals are subject matter experts in their field of study. They are also represented as the minority in the food industry. Most associates in the food industry are business, finance or marketing experts who are intimidated by those who work in the various fields of food science, many times speaking different languages. It is important for those working in food science to learn the business language that will allow for appropriate funding of programs and initiatives that will ensure safe and wholesome food for the customers in which these companies serve. This symposium is designed to take the scientific language of food safety and quality and translate it into business speak that the majority of business, finance and marketing associates will understand.

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S40 Future Challenges in Food Safety: An International Perspective

JARRET STOPFORTH, Campbell Soup Company, Camden, NJ, USA
BOBBY KRISHNA, Dubai Municipality, Dubai, United Arab Emirates
BERNADETTE FRANCO, University of Sao Paulo, Sao Paulo, Brazil
DEOG-HWAN OH, Kangwon National University, Chunchon, South Korea

Globalization and international trade has significantly impacted the way food is processed and distributed, and presents many new food safety challenges on an international scale. Current and imminent food safety issues and related challenges include (1) the need to control established and emerging foodborne pathogens, especially those with (2) increased virulence and (3) low infectious doses and (4) pathogens resistant to antibiotics and (5) food-related stresses. Additional pathogen-related concerns include (6) animal manure treatment/disposal and related environmental issues, (7) cross-contamination of food with enteric pathogens, (8) the need for real-time detection of pathogens at the point of contamination in the food chain, (9) foodborne illness surveillance and food attribution activities, and (10) food safety management through the development of risk assessment-based food safety objectives. Other food safety issues which need to be addressed include (11) hazards related to food additives, (12) chemical residues, (13) naturally occurring toxins, (14) intentional contamination, (15) the safety of organic and natural products, (16) hazards related to unconventional agents such as Bovine Spongiform Encephalopathy (BSE), or (17) unwanted byproducts of industrial processes and waste incineration, (18) heavy metal food-contamination via air and water pollution, (19) the safety of food derived from genetically modified organisms, (20) food product identification and traceability, (21) national and international regulatory inspection harmonization, and (22) international bioterrorism. This symposium will provide an international perspective and identify areas of mutual interest between countries, thereby strengthening international working relations.

S41 Using Nanotechnology for Improved Food Safety Testing in Food Industry

SAM NUGEN, University of Massachusetts-Amherst, Amherst, MA, USA
NITIN NITIN, University of California-Davis, Davis, CA, USA
ANDRE SENECAL, U. S. Army Natick RDE Center, Natick, MA, USA
VIVIAN CHI-HUA WU, University of Maine, Orono, ME, USA

The purpose of this symposium is to introduce food safety professionals to recent advances in nanotechnology which are being applied toward food safety. The contributions of nanotechnology toward food safety include new methods to both isolate and detect pathogens and toxins. Nanotechnology has enabled better sensitivity and lower limits of detection in the development of sensors for food pathogens, environmental toxicants, water parasites, etc. As contaminated foods continue to be problematic in the food industry, food safety professionals need to take full advantage of developing technologies which could provide faster, more sensitive or portable testing. This symposium will introduce several examples of nanotechnology being developed for increased food safety. Time will be given at the conclusion of the talks to allow audience members to ask the panel members questions and learn how these advances may soon benefit their business.

S42 *Bacillus cereus*: Heat Resistance and Psychrotrophy for Better Life in RTE Foods

REGINALD BENNETT, U.S. Food and Drug Administration, College Park, MD, USA
IRENE LUGOVAZ, Health Canada, Longueuil, QC, Canada
FREDERIC CARLIN, Inra-UMR, Avignon, France
PAUL IN 'T VELD, Food and Consumer Product Safety Authority, Endhoven, The Netherlands

The phylogenetic structure of *Bacillus cereus* sensu lato was recently resolved in seven major groups. The described genetic groups show clear differences in their clinical significance, in their ranges of growth temperature, of pH and of water activity and in their resistance to heat. Symptoms due to cereulide can vary considerably from very mild symptoms (vomiting) to very severe (death). Through all these characteristics, *B. cereus*group is of major concerns in ready-to-eat food risk management, while these RTE foods are praised by the consumers of the current century! This highlights the need to focus on the (re)emergence of this pathogen, and to consider whether these new food habits have created new niches for *B. cereus*, or whether adaptation has occurred. A range of topics will be covered, including the biodiversity, the prevalence of *B. cereus* in RTE foods, the pathogenic mechanisms and the regulation criteria. The phylogeny and the associated phenotypes will be presented. This phylogeny is of particular importance towards the evaluation of the potential risk associated with the *B. cereus* group strains. The occurrence of the cereulide encoding genes was recently investigated, through new advances in analytical methods. An overview of the collected data from starch-rich foods associated or not to food poisoning will be presented. At least, the epidemiology and the recent regulatory concerns with *B. cereus* contamination will be presented, highlighting the harmonization initiatives in North America. The presentations will produce perspectives on increasingly critical issues, and discussions on new strategies for better control.

S43 Fifty Years of Mycotoxins: A Retrospective and Prospective Examination

AILSA HOCKING, CSIRO, Sydney, Australia
NAI TRAN-DINH, CSIRO, Sydney, Australia
MARTA TANIWAKI, Instituto de Tecnologia de Alimentos, Campinas, Brazil
JOHN PITT, CSIRO, Sydney, Australia

It has been 50 years since the identification of the mycotoxin aflatoxin and, arguably, the establishment of the field of mycotoxicology. Over that time we have obtained a great deal of understanding about these insidious poisons that are produced as secondary metabolites of common fungi when they grow in foods and feeds. Mycotoxins cause acute sickness, cancer, kidney failure and other effects including death in humans, and poor performance, sickness or death in domestic animals. Additionally, mycotoxins have a profound economic impact on global agriculture and food security. This symposium will examine the major mycotoxins, including aflatoxin, fumonisins, trichothecene toxins and ochratoxin A, and highlight the multifaceted effects of mycotoxin contamination in foods and feeds. Speakers will reflect on mycotoxin research, intervention strategies to control mycotoxin contamination and visions for the future impacts of mycotoxins on global food safety and food security.

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Roundtable Abstracts

RT1 Current Controversies in Food Safety

W. PAYTON PRUETT, The Kroger Company, Cincinnati, OH, USA
BETSY BOOREN, American Meat Institute Foundation, Washington, D.C., USA
GLENN SONGER, The University of Arizona, Tucson, AZ, USA
BRANDI LIMBAGO, Centers for Disease Control and Prevention, Atlanta, GA, USA
DAVID ACHESON, Leavitt Partners, Glenelg, MD, USA
JENS KIRK ANDERSEN, Technical University of Denmark, Copenhagen, Denmark

This interactive roundtable is intended to engender lively discussion of important topics in food safety. It is assumed audience participants will have a basic understanding of the unresolved issues surrounding the topics in the roundtable. The session will cover three topics: "Should pasteurization of all raw ground meat and poultry be required prior to being offered for retail?"; "Is *Clostridium difficile* colitis a foodborne disease?"; "Should *Listeria monocytogenes* in low moisture foods be considered an adulterant?" Each topic will include a 9-minute presentation in support of (PRO) followed by a 9-minute presentation in opposition of (CON) to the proposed question. Each speaker will have 3 minutes for extemporaneous rebuttals. A 6-minute question/answer session will then follow to allow for audience participation. The session is intended to be informative, lively and humorous.

RT2 Microbiological Safety of Chilled ESL, Acidified, and Low-/High-acid Beverage Products

ALBERT ELBOUDWAREJ, Belkin International, Los Angeles, CA, USA
GLENN BLACK, Grocery Manufacturers Association, Washington, D.C., USA
FRED BREIDT, U.S. Department of Agriculture-ARS, Raleigh, NC, USA
NATHAN ANDERSON, U.S. Food and Drug Administration, Summit-Argo, IL, USA
CARRIE FERSTL, The National Food Lab, Livermore, CA, USA

While the global beverage industry continues to display an enviable microbiological safety record, it faces difficult challenges in defining methods and criteria for microbiological safety validation and verification, particularly for chilled extended-shelf-life (ESL) and high-acid products. These challenges are compounded by new regulatory requirements promulgated by the U.S. Food and Drug Administration (FDA), such as the Draft Guidance Document for Acidified Foods (released in September, 2010) and the impending Food Safety Modernization Act (FSMA)-driven requirements (final rule due July, 2012) pertaining to hazard analysis and preventive controls. The draft guidance document will likely force reclassification of many beverages into the acidified-foods category, which is governed by FDA regulations under the provisions of 21CFR114, and which requires application of a thermal process. As part of these requirements, many processors currently using ambient-fill technology will be forced to conduct rigorous challenge studies to demonstrate that microbial pathogens of significance die quickly if present in the final product. The FSMA regulation will impose mandatory preventative controls, with written implementation plans, on food facilities.

This roundtable will address the new U.S. regulatory requirements impacting domestic and international beverage manufacturers, as well as scientific strategies that could be employed to assure microbial safety and satisfy regulatory requirements. Subject matter experts will offer their perspectives on microbiological safety issues, including: verification of adequate hermetic seal on plastic beverage bottles; impact of acidification procedures on the survival of pathogens in high-acid environments at different storage temperatures; and thermal process and filler validations (for high-acid, refrigerated, and low-acid beverages), including proper identification of target microbial pathogens and potential surrogates to be used in validation strategies.

RT3 China – Food Safety for an Integrated World

PETER BEN EMBAREK, World Health Organization Office, Geneva, Switzerland
XIUMEI LIU, Ministry of Health, Beijing, China
LEON GORRIS, Unilever, Shanghai, China

Food safety within People's Republic of China in a globally traded food supply is a cause for concern among food safety professionals. This roundtable is meant to discus initiatives that are currently being performed and sharing, discussing, and collaborating on best practices for food safety within U.S. and China. The goal of this roundtable is to facilitate a technical discussion among the roundtable participants that may improve food safety efforts and future collaborations. Participants in proposed roundtable will intimately discuss the technological challenges for food safety within China and future collaborations needed on scientific initiatives.

RT4 Zero Risk Policies in a Non-zero Risk Environment

BARBARA CASSENS, U.S. Food and Drug Administration, Alameda, CA, USA
CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, Washington, D.C., USA
ROBERT BUCHANAN, University of Maryland, College Park, MD, USA

The concept of this roundtable is how regulators, growers and consumers are to deal with food safety risk in fresh produce, when produce is grown in the open, where only some risks are controllable and contamination is likely to occur at some low but non-zero level. Consumers expect all fresh produce to be pathogen free, and regulators acknowledge that some fresh produce risks are uncontrollable but still take regulatory action on any pathogen detection.

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RT5 Where Do We Go from Here: Discussion of Evidence-based Approaches to Education around Fresh Produce Safety

DIANE DUCHARME, North Carolina State University, Kannapolis, NC, USA
ELIZABETH BIHN, Cornell University, Geneva, NY, USA
KEITH SCHNEIDER, University of Florida, Gainesville, FL, USA
BETH BLAND, Georgia Fruit and Vegetable Growers Association, LaGrange, GA, USA
JAMES GORNY, U.S. Food and Drug Administration-CFSAN, Fulton, MD, USA
MICHAEL VILLANEVA, California Leafy Green Marketing Agreement, Sacramento, CA, USA

Although many industry partners and food safety educators had focused on transferring knowledge of good agricultural practices (GAPs) to producers, wholesalers and their employees for over a decade, high profile outbreaks (*Escherichia coli* O157 associated with spinach in 2006 and *Salmonella* associated with peppers in 2008) have raised the profile of these risks. This attention has resulted in increased focus from regulators and the market on implementation of practices. Questions remain about most effective methods (including messages and media) and acceptable measures of effectiveness of training programs. A discussion of barriers, success stories and differing needs of subset target audiences can better create collaborations and strengthen the quality of outputs.

The objective of this session is for the audience and panel members to share evidence-based approaches and lessons learned from program implementation, evaluation and barriers to success within this environment. Through prompts panel members will be asked to share best practices in education material design, implementation and evaluation.

This true roundtable will be a moderated discussion. Each of the six panel members will be allotted three minutes to introduce themselves and set the stage for the discussion. Participants will be provided with a selection of questions ahead of time focusing on barriers, successes and next steps. The audience will also select the direction of the discussion through the use of real-time feedback clickers.

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International Association for Food Protection®

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

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

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

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RT1 Current Controversies in Food Safety

This interactive roundtable is intended to engender lively discussion of important topics in food safety. It is assumed audience participants will have a basic understanding of the unresolved issues surrounding the topics in the roundtable. The session will cover three topics: "Should pasteurization of all raw ground meat and poultry be required prior to being offered for retail?"; "Is *Clostridium difficile colitis* a foodborne disease?"; "Should *Listeria monocytogenes* in low-moisture foods be considered an adulterant?" Each topic will include a 9-minute presentation in support of (PRO) followed by a 9-minute presentation in opposition of (CON) to the proposed question. Each speaker will have three minutes for extemporaneous rebuttals. A 6-minute question/answer session will then follow to allow for audience participation. The session is intended to be informative, lively and humorous.

W. PAYTON PRUETT, JR., The Kroger Company, Cincinnati, OH, USA

Should Pasteurization of All Raw Ground Meat and Poultry be Required Prior to Being Offered for Retail – The Con Perspective

BETSY BOOREN, Director, Scientific Affairs, American Meat Institute, Washington, D.C., USA

It should not be mandated that all raw ground meat and poultry be pasteurized prior to being sold at retail. The abundance of food in the U.S. allows consumers to have the ability to make food decisions based on their dietary needs, budget constrictions, and ethical and personal beliefs. Mandatory pasteurization of raw meat and poultry products removes that choice from the customer at a time where consumers demand more knowledge of where and how their food is produced. Mandatory pasteurization of raw meat and poultry products would also be detrimental to local sourcing of foods, i.e., farmers markets and local butchers, due to high capital investment and necessary scientific and technical knowledge needed to properly pasteurize the product. The local food sourcing accounted for 4.8 billion dollars of sales in 2008 (ERR-128, 2011); a figure that will continue to increase. Unlike other raw commodities, raw meat and poultry products typically will undergo some form of thermal treatment prior to consumption. If the thermal treatment is applied in a manner that meets U.S. Department of Agriculture's recommendations, the customer will enjoy a safe and nutritious product. Pasteurization of raw meat and poultry at retail should be voluntary to meet the need of the consumer as they decide the best product for their family and lifestyle. The diversity of the retail market allows for such a choice and the success or failure of those products will be decided at the checkout lane.

Clostridium difficile: The Latest Bad Bug and Coming on Strong, But from Where?

J. GLENN SONGER, Dept. of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ, USA

Clostridium difficile has been a recognized human pathogen for > 30 years. More recently, *C. difficile* has emerged as a common finding in enteric disease of neonatal pigs and calves. Genotypes of the organism from pigs and calves [primarily ribotype 078/toxinotype (TT) V] are becoming increasingly common in human *C. difficile*-associated disease, especially community-associated cases (i.e., those not originating in a healthcare facility and apparently not associated with antimicrobial use). Evidence derived from microarray-based comparative phylogenomic analysis suggests that strains causing human infections originate in animals. It is reasonable to hypothesize that, if these human strains originate in food animals, transmission may be by way of handling or consumption of retail meats. Retail meats were examined by bacteriologic culture as a test of this hypothesis. Toxigenic *C. difficile* was isolated from > 42% of retail meats intended for human consumption. The current human epidemic strain (ribotype 027/toxinotype III) accounted for 27% of isolates, obtained from pork braunschweiger, beef summer sausage, ground beef, ground pork, and pork chorizo. Examination by pulsed-field gel electrophoresis (PFGE) revealed that these were, in most cases, 88.9–100% related to human isolates. The majority (73.0%) of isolates from foods were ribotype 078/ TT V, from ground beef, ground pork, pork braunschweiger, pork chorizo, pork sausage, and ground turkey. These data support a contention that domestic animals, by way of retail meats, may be a source of *C. difficile* for human infection.

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BRANDI LIMBAGO, Centers for Disease Control and Prevention, Atlanta, GA, USA

DAVID ACHESON, Leavitt Partners, Glenelg, MD, USA

Should *Listeria monocytogenes* in Low-moisture Foods be Considered an Adulterant?

Birgit Norrung and JENS KIRK ANDERSEN, Technical University of Denmark, Copenhagen N, Denmark

Listeriosis in humans is a severe disease that primarily affects the elderly, people with compromised immune systems, pregnant women and the unborn child, causing bacteremia, systemic infections and miscarriages. In 2010, the total number of recorded human cases in the EU was 1601 corresponding to 0.35 cases per 100,000 inhabitants. *Listeria monocytogenes* is an ubiquitous organism in nature, and raw foods like milk, meat and vegetable may be contaminated with *L. monocytogenes* in low levels. In addition, from time to time, *L. monocytogenes* can also be found in both low- and high-moisture ready-to-eat foods. The foods which are associated with transmission of listeriosis are ready-to-eat foods that support growth of *L. monocytogenes*. Growth of *L. monocytogenes* is a function of the type of food, i.e., water activity, pH, storage time and temperature. The presentation includes short information on the characteristics of *L. monocytogenes*, dose/response relationships, occurrence in different food products and conclusions from risk assessments and considerations of risk management options. Published risk assessment concludes that most listeriosis cases are due to foods with the number of *L. monocytogenes* markedly above 100 per g.

We conclude that most low-moisture foods will not support the growth of *L. monocytogenes* within the shelf-life and therefore, *L. monocytogenes* should not be regarded as an adulterant in these foods.

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S5 Today Their Problem – Tomorrow Ours: Impact of International Trade on Food Safety

A greater knowledge of different kinds of organisms based on region of origin is warranted based on increased volumes of international trade in foods. New pathogens, new food products and the growing volume of imported foods are an increasing challenge to food safety. What unusual types or sub-types of pathogens might be associated with various international regions? Are these pathogens associated with foods? Import of a variety of food products and ingredients into the U.S. continues to increase. This symposium will explore unusual pathogens or pathogen subtypes (*Salmonella* serotypes and *Escherichia coli* serotypes) which might be encountered in imported foods from different parts of the world (Asia, Australia, South America and Eastern Europe) and what unusual foodborne disease outbreaks occur in different regions.

Pathogens in the International Food Supply – Why a Broader Perspective is Needed

MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA

Imported foods and ingredients have the potential to introduce, into a given country, unusual and atypical pathogens that can be transmitted through foods. A global understanding of viral, bacterial and parasitic pathogens as well as unusual pathogens (e.g., prions) is needed to allow industry and public health agencies to address potential food safety hazards associated with imported foods. In particular, many zoonotic pathogens that may not be well recognized in an importing country may pose a public a health hazard if present in imported foods. A lack of awareness of some of these pathogens, combined with a lack of diagnostic methods, compounds the potential economic and public health implications of these pathogens. For example, some zoonotic viruses, such as Ebola virus, have at least the potential to be transmitted through the foodborne route. In addition to unusual pathogens, pathogen strains or subtypes with unique or atypical phenotypic characteristics may also be introduced through imported foods. These unusual pathogen strains may not only cause outbreaks with unusual epidemiological features and atypical symptoms, but may also be difficult to detect with standard methods that are in use in a given country, particularly if these methods have not been validated with pathogen strains from other parts of the world. For example, the prevalence of different *Escherichia coli* strains that can cause hemorrhagic uremic syndrome appears to differ considerably between different parts of the world.

How International Surveillance of Foodborne Infections is Performed: The Role of WHO-International Health Regulations (IHR), the WHO International Food Safety Authorities Network (INFOSAN), the WHO Global Foodborne Infections Network (GFN) and PulseNet International

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

The role of four international networks/initiatives, each of which has important functions in the detection and response to international foodborne outbreaks and other emergencies, is presented here:

The IHR is the WHO alerting system for any public health emergency, infectious or non-infectious, with the potential to cross borders and threaten public health. It is a legally binding instrument, and therefore provides the legal framework for WHO's response to international public health emergencies. Foodborne infections are a fraction of the problems dealt with under IHR.

INFOSAN is the global network of national food safety authorities, but it also has participation from public health authorities. The network monitors different information resources and assesses, verifies and shares information about food safety events in collaboration with its members.

GFN enhances national and regional capacity to conduct integrated surveillance of foodborne infections. The network is open to food safety professionals in all links in the farm to fork continuum. Its activities include laboratory and epidemiology training, an external quality assurance program, reference testing and facilitating food safety surveillance projects.

PulseNet International is the global network of national and regional networks dedicated to molecular subtyping for foodborne disease surveillance. It works by building capacity for the surveillance and using that capacity to detect and investigate outbreaks of bacterial foodborne infections.

Conclusions: At the global level the IHR mandates that each country build capacity to detect and respond to public health emergencies of potential international importance. INFOSAN facilitates the surveillance and response to food safety emergencies, and GFN and PulseNet International facilitate building of such surveillance capacity, enabling rapid and efficient response to international outbreaks of foodborne infections.

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Salmonella, Unusual Serovars in Southeast Asia – Today Our Problem Due to Trade and Travel

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The majority of human *Salmonella* infections are caused by a limited number of the more than 2,600 *Salmonella* serovars described to date, and the prevalence of specific *Salmonella* serovars differs by geographical region. Globally, *Salmonella* serovars Typhimurium and Enteritidis are the most commonly reported causes of human salmonellosis, while in Southeast Asia other serovars seem to dominate. Several studies of non-typhoidal *Salmonella* from Thailand and Southeast Asia have shown a high prevalence of unusual *Salmonella* serovars such as *Salmonella* serovars Stanley, Weltevreden, Rissen, Kedougou, Hvittingfoss, Choleraesuis, Schwarzengrund, and Corvallis.

The differences in serovar distribution in Southeast Asia compared to the Western world complicate outbreak investigation, why implementing source attribution might reveal the true nature of the serovars associated with trade and travel to Southeast Asia. In 2010, data from the Danish source attribution system showed that 10% of human *Salmonella* infections in Denmark were associated with imported meat, 47% were associated with international travel and 18% were associated with domestically produced meat products. A lot of these cases associated with either travel to Southeast Asia or consumption of imported food from the region have indicated transmission of the same unusual *Salmonella* serovar dominating the region.

There is still a lack of knowledge regarding the global epidemiology of *Salmonella* in both Southeast Asia and other parts of the world. It seems as we are in a transition phase where well-known *Salmonella* serovars disappear to favour more unusual serovars only known sporadically from developing countries or distant destinations and regions. There is a global need for implementing timely, systematic, integrated laboratory-based surveillance for *Salmonella.* Surveillance, in combination with extensive collection of epidemiological data, are needed to diminish the worldwide burden of human salmonellosis by implementing targeted prevention and intervention strategies.

Global Food Trade and Emerging Foodborne Pathogens: The Example of *Escherichia coli* O104

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Innovation in the food sector is driven by consumer expectations: Pleasure, health, convenience and ethics. This implies enforcement of R&D programs by the food industry and the use of an external market for the supply of raw materials. As a matter of fact, the figure of the EU import in 2010 was 35,124 € million, with a significant income from emerging countries in South America, Asia and Africa. Enlargement of the food market to developing countries opened the way to the diffusion of pathogens that either disappeared from industrialized countries or represent emerging variants of known pathogens, challenging the public health systems and having devastating effect on the consumers' confidence. The recent Shiga toxin-producing *Escherichia coli* (STEC) O104 crisis in the EU is paradigmatic. STEC are zoonotic pathogens characterized by a very high within-group heterogeneity. More than 200 STEC serotypes have been described so far, but those causing severe disease in humans represent a restricted group of serogroups, such as O157, O26, O111, O103 and O145. STEC O104 is an Enteroaggregative *E. coli* (EAEC) that developed the capability to produce Shiga toxin (*Stx*). EAEC infections are common in developing countries where the pathogen have an inter-human circulation with an oral-fecal route. EAEC do not come into contact with *Stx*-phages, which are common in the ruminants' gut and in the environment contaminated by ruminants' feces but, under certain circumstances, it is possible that EAEC released in surface waters encounter bacterial strains of animal origin, making possible the exchange of genetic material, including the *Stx*-phages. Such a scenario brings the attention to food products, particularly of vegetable origin, produced in developing countries, where the environment represents an alternative reservoir for unusual pathogenic STEC emerging from the combination of characters from diverse *E. coli* pathotypes.

Foodborne Viruses – What Else is Out There?

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The study of foodborne illness has historically focused on gastroenteritis and bacteria. In later years, viruses such as hepatitis A, norovirus, and rotavirus have been recognized as major causes. Advances in diagnostic platforms as well as in epidemiologic approaches continue to reveal new viral etiologies, such as hepatitis E, sapovirus, astrovirus, human parechovirus, Aichivirus, and human bocavirus. Of increasing concern are "exotic viruses" found at the zoonotic interface and that cause syndromes other than gastroenteritis. Although the primary reservoirs are usually wild animals, livestock may serve as intermediate hosts transmitting these viruses on to humans, even potentially resulting in pandemics, such as the 2003 pandemic of SARS-coronavirus that, although maintained in nature in bats, likely first entered into humans through exposure to animals in live markets in China. Nipah virus in pigs and avian influenza virus in chickens are further examples of zoonotic viruses that may work their way into the food chain. Although the highest risk of zoonotic virus infection is usually to livestock workers as occupational exposure, disease from direct consumption of contaminated food cannot be ruled out, and has been demonstrated in a few cases. Although not shown to cause disease in humans, Ebola Reston virus, which has been found in pigs in the Philippines, simian foamy viruses found in nonhuman primates that are often hunted as "bush meat" in Africa, and a variety of prions found in wild cervids (hoofed ruminant mammals) that are often the target of hunters in North America, bring up special concerns.

Parasites – Southeast Asia

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S11 *Salmonella* in Low-moisture Foods: A Continued Challenge

Human salmonellosis has remained a considerable challenge for the U.S. food industry, regulatory and public health agencies over the last decade. This challenge is illustrated by the fact that the annual incidence of human salmonellosis cases in the U.S. has not decreased over the last decade, despite the fact that the prevalence of a number of illnesses caused by a number of other foodborne pathogens has decreased considerably over the same time frame. For a variety of reasons, control of *Salmonella* in low-moisture foods represents a particular challenge. This symposium will include an introductory overview presentation on the challenges of controlling *Salmonella* in low-moisture foods as well as the outlook for improved and novel approaches to address this issue. Recognizing the challenges of controlling *Salmonella* in low-moisture foods, the ILSI North America Technical Committee on Food Microbiology has, over the last years, funded a number of research projects in this area. The rest of this symposium will focus in providing attendees with new information and science on *Salmonella* in low-moisture foods, resulting from these on-going research projects.

Salmonella in Low-moisture Foods: Challenges and Potential Solutions

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Inactivation of *Salmonella* on Raw Nuts Using Low-energy X-ray

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Microbial safety of raw almonds and other nuts has been questioned due to a series of recent salmonellosis outbreaks linked to these products. The 2007 mandate for a treatment to reduce *Salmonella* populations > 4 logs on raw California almonds prior to shipment has created industry-wide demand for various strategies to achieve this goal without adversely impacting organoleptic characteristics of the end product. Low-energy X-ray, one of the FDA-approved food irradiation technologies, has shown good efficacy and wide-reaching potential. Since the microbiological safety of dry products is primarily dictated by moisture content, this study investigated the impact of water activity on efficacy of low-energy X-ray for inactivation of *Salmonella*. Shelled raw whole Nonpareil almonds and California walnuts were inoculated with *Salmonella*, conditioned to target a_w (0.2~0.8), irradiated, and enumerated on a non-selective/differential plating medium. Greater efficacy of low-energy X-ray was seen for *Salmonella* on almonds (D_{10} = 0.226–0.431 kGy) than walnuts (D_{10} = 0.474–0.930 kGy) at all water activities with *Salmonella* inactivation changing non-monotonically with water activity. No significant differences ($P > 0.05$) in sensory characteristics were seen between the control and samples irradiated to achieve a 5 log reduction in *Salmonella*; however, differences were seen for walnuts. Based on these findings, low-energy X-ray irradiation technology appears to be a promising pathogen reduction strategy for certain types of nuts and potentially other dry products.

Thermal Inactivation and Survival of *Salmonella* in Food as a Function of Water Activity and Fat Level

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The Objective of this study was (i) to evaluate how a_w and fat affect the thermal destruction of *Salmonella* Tennessee, *S.* Typhimurium DT104 and *E. faecium* in model food systems made from peanut flour, (ii) to evaluate how *Salmonella* survival over time is affected in the various combinations of water activity (a_w) and fat in a model food system after the cells have been thermally stressed, but not destroyed. *E. faecium* was more heat resistant than *S.* Tennessee and *S.* Typhimurium DT 104 in all four peanut pastes at extreme a_w (0.3 – 0.6) and fat levels (47 – 56%). *S.* Tennessee was slightly more heat sensitive than *S.* Typhimurium DT 104 in all peanut pastes, except for F_{16} (56 % fat and a_w = 0.6). Maximum inactivation was achieved in peanut pastes with highest fat level and lowest a_w (F_{13}), for all three organisms. Maximum destruction of *S.* Typhimurium DT 104 was reached in the formulations with highest fat level (56%) at both 0.3 and 0.6 a_w, implying that fat level might be a more critical factor than a_w in heat inactivation of this organism; a similar pattern was observed for *E. faecium*. More upwardly concave and tailing of the inactivation curves of all tested organisms heated in peanut pastes was observed at 0.6 than 0.3 a_w level. After one month storage at 20 ± 1°C the decline of *S.* Tennessee, *S.* Typhimurium and *E. faecium* was up to 1.1, 1.8 and 0.3 log CFU/g, respectively, in the tested pastes.

ILSI Symposium

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Influence of Water Mobility on Persistence of *Salmonella* in Low-moisture Foods

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The presence of *Salmonella* in low-moisture foods has caused numerous disease outbreaks. *Salmonella* will persist for long periods of time in these foods, but little is known about how the water component of the food affects its survival. The interaction of water with bacterial cells is a key factor in microbial inactivation. Water in dry foods has limited mobility and therefore limited ability to interact with bacterial cells. Mobility of water at the molecular level can be estimated using proton NMR, whereas the ability of water to equilibrate to a vapor state is associated with water activity. In this study, whey protein was manipulated by pH adjustment to achieve three different subunit configurations with resulting changes in the protein's ability to interact with water. Samples were equilibrated to various water activities, inoculated with *Salmonella* and stored at temperatures ranging from 20 to 80°C. Protein configuration was associated with different water mobilities in samples equilibrated to a_w 0.37, 0.47 and 0.59, but not a_w 0.16 and 0.28. Inactivation of *Salmonella* increased with increasing water activity and temperature. Increased water mobility was associated with increased survival of *Salmonella* in samples treated at 80°C but had no significant effect in samples held at 70°C or lower. Data indicate that inactivation of *Salmonella* in dry foods by heat treatment is a complex phenomenon that may not be directly associated with water mobility at ambient temperatures.

Improved Process Validation Strategies for *Salmonella* Inactivation on Low-moisture Food Products Subjected to Thermal Pasteurization Processes

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Low-moisture food pasteurization is significantly affected by water activity (a_w) and process conditions; however, processors currently have no way to know which validation method yields the most accurate or reliable results. To address these critical gaps, the specific objectives were: (1) To quantify the accuracy and repeatability of multiple validation methods for thermal pasteurization of almonds, and (2) To develop and test an improved thermal inactivation model accounting for the effect of a_w and process humidity. Overall, the project entailed: (1) inoculation of almonds with *Salmonella* Enteritidis PT30 or *Enterococcus faecium* (NRRL B-2354), (2) a_w equilibration (~0.25 to 0.84), (3) dry or moist-air heating treatments (~121 to 177°C; ~0 to 70% moisture by volume; target lethality 4 to 5 log reductions) in a pilot-scale impingement oven, (4) measurement of surface temperature via multiple means, (5) recovery/enumeration of survivors, (6) quantification of lethality variability and error (vs. actual *Salmonella* lethality), and (7) development of an improved lethality model. In terms of accuracy, validations based on the surrogate (*E. faecium*) and the modified model yielded comparable prediction biases (~0.5 log CFU/g) and root mean squared errors (~1.3 log CFU/g); however, the model-based validations yielded significantly better repeatability (~35–60% lower) than the biologically-based validations. Although the traditional inactivation model (D, z) worked sufficiently well for dry heat, it yielded unacceptable results for moist heat (bias and RMSE ~3.6 and 3.9 log CFU/g, respectively). Overall, product a_w, process humidity, and model choice all impact accuracy and repeatability of pasteurization validations for dry products, which must be considered when choosing and applying a specific validation strategy.

Using Limited Data Sets to Assess *Salmonella* Risk in Low-moisture Foods

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Peanuts and peanut-containing products have been linked to at least seven salmonellosis outbreaks around the world in the past two decades. In response, the Technical Committee on Food Microbiology of the North American Branch of the International Life Sciences Institute collaborated with the American Peanut Council to convene a workshop to develop a framework for managing risk in low-moisture food commodities where large data sets are unavailable (using peanuts as the example). Workshop attendees were charged with answering questions regarding: appropriate statistical and scientific methods for setting log reduction targets with limited pathogen prevalence and concentration data; suitable quantities of data needed for determining appropriate log reduction targets; whether the requirement of a 5-log reduction in the absence of data to establish a target log reduction is appropriate; and what targeted log reduction would protect public health. This report concludes that the judgment about sufficient data is not solely scientific, but is instead a science-informed policy decision that has to weigh additional societal issues. The participants noted that modeling efforts should proceed with sampling efforts, allowing one to compare various assumptions about prevalence and concentration and how they are combined. The discussions made clear that data and risk models developed for other low moisture foods like almonds and pistachios may be applicable to peanuts. The workshop participants were comfortable with the use of a 5-log reduction for controlling risk in products like peanuts when the level of contamination of the raw ingredients is low (< 1 CFU/g) and well controlled, even when limited data are available. The relevant stakeholders from the food safety community may eventually conclude that as additional data, assumption and models are developed, alternatives to a 5-log reduction might also result in the desired level of protection for peanuts and peanut products.

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Technical Abstracts

T1-01 The Role of Aggregative Fimbriae and Cellulose in the Persistence of *Salmonella* Typhimurium on Tomatoes

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Developing Scientist Competitor

Introduction: The rise of produce-linked salmonellosis outbreaks has directed attention towards the persistence of *Salmonella* spp. A complex phenotype of *Salmonella* spp., known as *rdar* (red dry and rough), has been linked to increased resistance to desiccation and environmental stress. Thin aggregative fimbriae, cellulose, and production of other extracellular polysaccharides mediate the *rdar* phenotype, as well as attachment and biofilm formation. The deletion of the aggregative fimbriae and cellulose biosynthesis genes results in a loss of the *rdar* phenotype. The comprehension of how aggregative fimbriae and cellulose alter the biofilm formation and persistence of *Salmonella* spp. on the tomato surface will indicate their role in their environmental fitness.

Purpose: The aim was to study the role of aggregative fimbriae and cellulose of *S.* Typhimurium on the tomato surface to further characterize the genetic basis of the surface interactions between *Salmonella* spp. and produce.

Methods: Analysis of the biofilm formation of *S.* Typhimurium (ATCC 14028) and *S.* Typhimurium mutants deficient in thin aggregative fimbriae and/or cellulose production were completed in both polystyrene and plant surface environments. Diluted solutions of the *Salmonella* strains in colonization factor antigen (CFA) media were incubated in 96-well polystyrene plates at 30°C for 24 hours. The plant surface assays involved soaking the plant segments in the inoculated CFA media for 24 hours at 30°C. After the 24 hours, the 96-well plates or the produce segments were stained with crystal violet. The biofilms were then solubilized with acetic acid and absorbance at 595 nm was recorded.

Results: The deletion of aggregative fimbriae and cellulose biosynthesis genes does significantly reduce the biofilm formation of *S.* Typhimurium on polystyrene and plant surfaces, indicating that both components play a role in environmental persistence.

Significance: The results support the importance of aggregative fimbriae and cellulose in the environmental fitness *S.* Typhimurium to the surface of tomatoes.

T1-02 Influence of Soil Type, Nitrogen Application and Microbial Community Composition on Survival of *Escherichia coli* O157:H7 under Organic and Conventional Spinach Production

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Developing Scientist Competitor

Introduction: Examining the relationship between soil physicochemical characteristics and survival of *Esherichia coli* O157:H7 (*Ec*O157) will provide a platform for the development of recommended farming practices (RFP).

Purpose: To evaluate the influence of soil type, nutrient availability and microbial community composition (MCC) on the survival of *Ec*O157 in the spinach rhizosphere.

Methods: Four soil types were inoculated with a mixture of two *stx*-minus *Ec*O157 under growth chamber, greenhouse and field conditions. Organic crops were fertilized by application of chicken pellets (8:1:1) at 90, 180 and 270 kg/ha to soil. Conventional soils received total nitrogen applications of 112, 224, and 336 kg/ha. Growth chamber inoculum was log 2.1 CFU/g soil and incubation temperatures of 15 and 25 °C. Greenhouse inoculum was log 2.3 CFU/g soil. Field inoculum was log 4.6 CFU/g sachet. Inoculations were done after sowing or at two true-leaves. Soil physicochemical analysis included total carbon, nitrogen, pH and electrical conductivity. Total MCC and diversity was estimated by using phospholipid fatty acid analysis (PLFA).

Results: Growth chamber-Greenhouse A significant positive correlation between the survival of *Ec*O157, soil clay content, and chicken pellet rate was observed ($P < 0.05$). Time to non-detection of *Ec*O157 was shorter in conventional than organic sandy loam soils (30 vs. 45 days, respectively). In conventional soils, nitrogen availability <224 kg/ha and 15 °C extended the survival of *Ec*O157 ($P < 0.05$). Conventional fields. No correlation was observed between N availability and pathogen survival; however, greater survival was observed in Sandy Loam than Loamy Sand soils (60 vs. 31 days, respectively ($P < 0.05$)). Overall, PLFA analysis indicated that greater microbial community diversity and biomass was associated with faster die-off of *Ec*O157, irrespective of soil type and farming practice.

Significance: Development of RFP intended to limit human pathogen persistence in cultivated soils should consider the soil physicochemical characteristics as a focal point for preventive controls or mitigations.

T1-03 Airborne Soil Particulates as Vehicles for *Salmonella* Contamination in Tomatoes

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Developing Scientist Competitor

Introduction: Several outbreaks of salmonellosis have been associated with the consumption of fresh tomatoes. *Salmonella* serotypes have been shown to survive in soil and may persist in the farm environment. However, tomato contamination routes in the field environment remain unclear.

Purpose: The potential of soil particulates as vehicles of contamination of tomato blossoms and fruits was examined.

Methods: *Salmonella* Newport (containing the pNSTrc-lux plasmid) was grown in TSB (24 h; 37°C) and centrifuged. The cell pellet was mixed with soil and dried (9 CFU/g of soil). Using compressed air, the *Salmonella*/soil mixture was delivered to tomato blossoms. One week after treatment, the blossoms were removed from the plant, pummeled in peptone water, and plated onto XLT4 Agar. Additionally, treated blossoms were imaged with a high sensitivity CCD camera to determine the location of bioluminescent *Salmonella*.

Results: When the *S.* Newport/soil mixture was applied using compressed air, 29% of blossoms were positive for pathogen presence. More than half of the positive blossoms (51%) developed into fruit that were also positive for the presence of *S.* Newport. Fruit and calyx tissue were

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positive for *S*. Newport presence even after surface sterilization. Biophotonic imaging revealed that *Salmonella*cells were transferred to the petals, stamen and pedicel of the blossom.

Significance: Highly contaminated airborne soil particles may be capable of transferring *Salmonella* to tomato blossoms. *Salmonella* presence in blossoms may result in contaminated tomato fruits.

T1-04 Influence of Soil Particles on the Survival of *Salmonella* on Plastic Tomato Harvest Containers

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Introduction: Produce-related outbreaks of foodborne illnesses is a fast growing trend in food safety. *Salmonella*contamination of fresh tomatoes can occur at the picking stage of the process, and the bacteria can survive on the surface of the plastic collection totes used to harvest tomatoes.

Purpose: To investigate if soil and humidity conditions alter *Salmonella*survival on plastic tomato collection totes.

Methods: Five produce-related, nalidixic acid-resistant strains of *Salmonella enterica*were standardized and mixed before being added to water containing 5% horse serum, 5% soil, or a mix of 5% horse serum and 5% soil. The non-supplemented standard inoculum was also tested. Tote coupons were steeped in the mixture and incubated at 20°C at 94%, 75%, 54% or 33% relative humidity (RH). Coupons were analyzed and quantified by colony forming units at five time points over 28 days.

Results: Analysis of variance indicated the addition of 5% soil to the mixture of bacteria significantly increased long term bacterial survival over the non-supplemented mixture ($P < 0.001$); survival was up to 4-5 log higher at all humidity levels, with the exception of 33% RH, where a 3-log increase in survival was observed. Survival at 33% RH was greater than at the higher humidity levels for all the inoculation variations tested ($P < 0.001$). No significant differences in bacterial survival were detected until 14 days post inoculation. Confocal microscopy confirmed that *Salmonella*cells were associated with soil components within the mixtures.

Significance: Results indicate improved and regular sanitation of plastic totes must be implemented to reduce the possibility of *Salmonella* contamination of harvested tomatoes. Adequate washing and removal of soil and other organic material may help reduce *Salmonella* survival.

T1-05 Influence of Poultry Litter and Dairy Manure on Persistence of Non-pathogenic *Escherichia coli* and *E. coli* O157:H7 Applied to Fields

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Introduction: Manure amendment of soils used to grow fresh produce can introduce pathogens which may persist and contaminate vulnerable commodities. Edaphic, environmental, and biological factors influence microbial survival differently.

Purpose: Determine the influence of manure type applied to field plots on survival of a multi-strain mixture of non-pathogenic *Escherichia coli (Ec)* and attenuated *E. coli*O157:H7 (attO157).

Methods: Seventy-nine uniformly-sized field plots grouped by manure type and randomized for low and high-inoculum applications were established with six manure-soil treatments: poultry litter, dairy manure liquids, dairy manure solids plus dairy manure liquids, dairy solids on organic and on conventional soil, and organic soil only. Three rifampicin-resistant (Rif^R) strains of *Ec* and two Rif^R strains of attO157 grown in dairy manure extract were sprayed onto plots as a single inoculum at ~5×10^6 CFU/m^2 (low) and 5×10^8 CFU/m^2 (high). Soil samples collected for 30 days post-inoculation were analyzed for viable *E. coli*by direct plating and/or MPN analysis.

Results: On day 0, average populations of both *Ec* and att0157 recovered from low treatments were 3.4 log CFU/g; whereas populations were 6.1 and 6.0 log CFU/g for *Ec* and attO157, respectively, in high treatments. After 14 days, *Ec*counts were 0.9 log CFU/g and 1.4 log CFU/g greater than attO157 counts, for low and high inoculum treatments, respectively. All *E. coli* survived at higher populations in poultry litter and dairy manure liquids compared to other manure treatments. Rapid population decreases for attO157 occurred in organic soil and dairy solids treatments.

Significance: Manure type can have a substantial influence on *E. coli* and *E. coli* O157:H7 survival, and should be taken into account when setting guidelines for fresh produce safety. Non-pathogenic, field-isolated *E. coli* were more persistent than attO157 in manure-amended soils at 30 days post-inoculation.

T1-06 Inactivation of *Escherichia coli* O157:H7 in Crop Soil by Amending with Fast and Slow Pyrolysis-generated Biochars

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Introduction: Biochar, generated by pyrolysis (*i.e.*, heating biomass under low-oxygen conditions), results in incomplete combustion, producing a fine, grainy, highly porous material, 200 – 1,000 µm in diameter. Benefits of biochar production include generation of bio-fuels, useful soil amendments for fertilizing crops and binding heavy metals, sequestering biocarbon, and reducing environmental gas emissions. Data also suggest that biochar-amended soil improves the phyto-beneficial microflora and depresses populations of human pathogens within the family Proteobacteria (*Salmonella, etc*.).

Purpose: To determine the influence of biochar on the survival of *E. coli* O157:H7 (EHEC) in crop soil.

Methods: Twelve types of biochar (slow pyrolysis or fast pyrolysis type) were generated in on-site reactors. A three-strain composite of EHEC (6 log CFU/g, final population) was added to autoclaved crop soil + 10% (final concentration) of one of 12 types of biochar. Samples were adjusted to a final moisture content of 17.6% and stored in sealed bags at 22 °C. Populations of EHEC were determined weekly up to five weeks.

Results: Fast pyrolysis-produced switchgrass (FP-SG) and FP horse litter (FP-HL) biochars inactivated 3.52 and 2.58 log CFU/g more EHEC than did control soil by day 14. By day 28, FP-SG, FP-HL, and FPpelleted-SG biochars all reduced EHEC to undetectable levels (< 1.2 log CFU/g) by direct plating versus control soil, which contained 5.45 log CFU/g of EHEC on day 28. FP oak biochar inactivated 3.1 log more EHEC than control soil on day 28, and at day 35, no EHEC was detected in FP-oak soil by direct plating. Four slow pyrolysis biochars all inactivated 2 log CFU/g more EHEC than the control soil by day 28, including SG (1 h at 500 °C), mixed hardwood pellets (MHP, 1 h at 500 °C), and MHP (30 min at 700 °C).

Significance: These results are the first to suggest that biochar amendments enhance the inactivation of EHEC in crop soil.

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T1-07 Effects of Temperature Differential and Immersion Time on Internalization of *Salmonella* Newport in Tomatoes

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Introduction: Foodborne illness outbreaks associated with *Salmonella enterica* have been traced back to tomatoes contaminated through bacterial attachment and possible internalization during post-harvest handling. However, no scientific information is available regarding the effect of current tomato dump tank handling conditions on *S. enterica*internalization.

Purpose: This study was conducted to determine the effect of immersion time and temperature differential between tomato pulp and wash solution on the internalization of *S. enterica*in tomatoes, and its distribution once internalized.

Methods: Freshly harvested mature green tomatoes held at 90°F (32.2°C) were immersed in water containing approximately 100,000 CFU/ml *S. enterica* Newport. Tomato variety (cv. BHN602 and Sun Bright), temperature differential (-30, -10, 0, 10°F) between tomato pulp and the inoculated wash solution, and immersion time (2, 5, 10, 15 min) were evaluated for their effects on *S. enterica* internalization, as well as the effects of these postharvest handling conditions on the infiltration depth. The incidence and cell populations were determined via culture enrichment and most probable number methods, respectively.

Results: The incidence and extent of *S. enterica* internalization was significantly impacted by the range of temperature differential and immersion time and their interactions. Significantly more severe pathogen internalization was observed with a 15-min immersion time than a 2-min immersion time. With a 15-min immersion time, significantly more pathogen internalization was observed with temperature differentials of -10 and -30°F than with temperature differentials of 0 and 10°F. However, with a 2-min immersion time, no significant difference for pathogen internalization was observed among the temperature differentials of 10 to -30°F under the testing conditions.

Significance: This study provides critical information for the tomato industry and the FDA to develop science-based food safety regulations and guidelines to prevent pathogen internalization.

T1-08 Impact of Organic Load on *Escherichia coli* O157:H7 Survival during Pilot-scale Processing of Iceberg Lettuce with Acidified Sodium Hypochlorite

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Developing Scientist Competitor

Introduction: Sodium hypochlorite is routinely used in flume tanks during leafy green processing to reduce bacterial pathogens both in the water and on the product. However, its efficacy continues to be questioned.

Purpose: The aim of this study was to assess the relationship between organic load and various physicochemical wash water parameters on the efficacy of sodium hypochlorite with or without two different acidifiers against *Escherichia coli*O157:H7 during pilot-scale production of fresh-cut iceberg lettuce.

Methods: Flume tank water containing 10% (w/v) blended iceberg lettuce and a commercial chlorine-based produce sanitizer (XY-12, Ecolab, St. Paul, MN) at a concentration of 50 ppm free chlorine (pH 8.1) was adjusted to pH 6.5 using two acidifiers - SmartWash (SW) (New Leaf Food Safety Solutions, Salinas, CA) or citric acid (CA). Sanitizer efficacy against a 4-strain, avirulent, GFP-labeled *E. coli* O157:H7 cocktail was then assessed in triplicate trials by processing dip-inoculated (5.4 kg) followed by uninoculated (3 x 5.4 kg each) heads of iceberg lettuce at 10 min intervals in a pilot-scale processing line from shredding through centrifugal drying with sanitizer-free water (pH 7.3) serving as the control. Shredded lettuce (25 g) and water samples (50 ml) were collected every 30 s from the 3.3-m flume tank along with additional water samples at 2-min intervals between batches. Wash water was also assessed for various physicochemical parameters, most importantly pH and oxidation/reduction potential (ORP). All samples were appropriately neutralized, diluted and surface-plated on TSAYE + ampicillin with or without membrane filtration to enumerate *E. coli*O157:H7.

Results: All three sanitizer treatments were more effective ($P < 0.05$) than water alone, with chlorine, chlorine + SW, and chlorine + CA respectively reducing *E. coli* O157:H7 populations 0.43, 1.54, and 1.58 logs on lettuce. Average *E. coli*O157:H7 levels in the water after processing were significantly lower ($P < 0.05$) using chlorine + CA (0.53 log CFU/ml) or chlorine + SW (-0.04 log CFU/ml) at pH 6.5 than chlorine alone (2.96 log CFU/ml) at pH 8.09. Oxidation/reduction potential was significantly higher using CA and SW ($P < 0.05$) than chlorine alone.

Significance: Both SmartWash and citric acid effectively increased the efficacy of sodium hypochlorite against *E. coli* O157:H7 populations in wash water containing a 10% organic load, thereby decreasing the extent of cross contamination. However, sanitizer use alone cannot ensure end product safety.

T1-09 Impact of Roller Type on *Salmonella* Transfer during Simulated Commercial Conveyance of Tomatoes

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Developing Scientist Competitor

Introduction: Different types of roller conveyors are used in the commercial tomato packinghouses with *Salmonella*cross-contamination of tomatoes occurring via direct contact with the surface of these rollers during product conveying.

Purpose: The goal of this study was to quantify the transfer of *Salmonella*from inoculated tomatoes to three different roller surfaces and then from inoculated roller surfaces to uninoculated tomatoes using a pilot-scale packing line.

Methods: Five lbs (2.3 kg) of greenhouse-grown red round tomatoes were dip-inoculated to contain *Salmonella* Typhimurium LT2 (avirulent) at 4 log CFU/g, air-dried for 2 h in a biosafety cabinet and then washed in sanitizer-free water for 2 min. Inoculated tomatoes were passed single file over a specially designed 1.5-m conveyor equipped with polyethylene, foam or brush rollers followed by 25 uninoculated tomatoes. Two tomatoes (~500 g) were collected after both 2 min of washing and roller conveying. All 25 uninoculated tomatoes were individually collected after conveying. Six polyethylene (118 cm^2), three foam (362 cm^2) and three brush roller (4 bunches of bristles) surface samples were collected using Kimwipes®before and after conveying the uninoculated tomatoes. Tomatoes were hand-rubbed in 100 ml of phosphate buffer in a Whirl-pak bag for 2 min and then surface-plated on Xylose Lysine Tergitol-4 agar (XLT-4) with or without 0.45 µm membrane filtration to quantify *Salmonella*. Surface samples were homogenized by stomaching in 15 ml of phosphate buffer for 1 min and then similarly examined for salmonellae.

Results: Regardless of the roller type, *Salmonella* populations on inoculated tomatoes did not significantly ($P < 0.05$) decrease during contact with the roller conveyors. After conveying uninoculated tomatoes over the product-inoculated foam rollers, all 25 previously uncontaminated tomatoes were cross-contaminated with *Salmonella* at >100 CFU/tomato. Using polyethylene rollers, 24 and 76% of the 25 tomatoes were cross-

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contaminated with *Salmonella* at 10 - 100 and 1 - 10 CFU/tomato, respectively. In contrast, only 8% of 25 tomatoes were cross-contaminated using brush rollers with *Salmonella* populations of 1 - 10 CFU/tomato. Overall, cross-contamination was greatest using foam ($P < 0.05$), followed by polyethylene ($P < 0.05$) and brush rollers ($P < 0.05$).

Significance: These findings, which clearly demonstrate the ability of *Salmonella* to transfer from roller conveyors to uncontaminated tomatoes during processing, are critical to the development of science-based transfer models for risk assessments.

T1-10 Enhanced Inactivation of *Salmonella*, *Escherichia coli* O157:H7 and Pseudomonas Biofilms Using Fresh Produce Washing Aid, T-128, on Cantaloupe Rinds with Chlorinated Wash Solutions

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Introduction: Cantaloupes have been associated with numerous outbreaks of foodborne pathogens in recent years. Survival of bacterial microorganisms on cantaloupe rinds is mainly attributed to its high contact with soil during growth, netted texture rind, and bacterial incorporation into biofilms on rinds. Consequently, development of highly effective disinfection methods is needed to improve safety of fresh cantaloupe.

Purpose: This study was conducted to evaluate the efficacy of chlorinated water (CW, with hypochlorous acid) solutions, with or without the washing aid, T-128, on inactivation of natural microbial flora, *Salmonella enterica* serovars Poona and Newport, *Pseudomonas fluorescens*, and *Escherichia coli*O157:H7 biofilms on cantaloupe rinds.

Methods: Biofilms were formed by spot-inoculation of pure- and co-cultured bacterial strains on cantaloupe rind surfaces, followed by 24-h storage at 22 °C. Cantaloupes were manually washed for 5 min or vigorously scrubbed with fruit brushes for 1 min in CW (200, 500, 800, 1000, or 2000 mg free chlorine (FC)/l at pH 5.0 or 2.8) with or without T-128. Cell populations on cantaloupe rinds were dispersed using intermittent pulsed-ultrasonication and stomaching, and enumerated using modified MPN or plating onto XLT-4 agar or petrifilms. Statistical analysis of data was performed using PROC Mixed procedure of SAS.

Results: In general, for natural microbial flora and bacterial biofilms, the FC (500-2000 mg/l) sanitizing effects were enhanced ($P < 0.05$) by approximately 1.0-2.0 log CFU/cm^2 when combined with T-128, especially in 2000 mg/l CW with T-128, by manually washing. An additional reduction ($P < 0.05$) of 0.7-1.0 log CFU/cm^2 of *S.* Poona or *E. coli*O157:H7 was observed in CW (500-1000 mg/l) with T-128 with the brush-scrubbing wash.

Significance: These results indicate that T-128 can aid in reducing pathogen viability in biofilms on cantaloupe rinds, and thereby can aid in reducing food safety risks associated with fresh cantaloupes.

T1-11 Inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on Strawberries by Sanitizing Solutions

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Introduction: A recent foodborne outbreak of *E. coli* O157:H7 in Oregon associated with the consumption of fresh strawberries highlights the need for effective sanitizing washes, suitable for the inactivation of pathogens on fresh produce.

Purpose: To screen sanitizing solutions for decontaminating *Escherichia coli* O157:H7 (EHEC) and *Salmonella enterica* serovars from strawberries.

Methods: Four serovars of *Salmonella* (serovars Saintpaul, Montevideo, Newport, and Stanley) and three strains of EHEC were grown individually in TSB + 100 ppm nalidixic acid (TSBN), washed by centrifugation and combined into a seven-strain inoculum. Strawberries were flooded with the inoculum three times and dried under a laminar flow hood for 2 hours. Strawberries were then washed for 2 min within respective sanitizing solutions at 21 °C, and diluted 1:2 (weight:volume) with Dey-Engley neutralizing broth. Strawberries were macerated with a hammer in filtered bags and pummeled in a stomacher for 2 min. Homogenate was plated on TSAN + 0.1% sodium pyruvate, incubated for 24 h at 37 °C, and colonies were enumerated.

Results: Inoculation levels were 7.07 log CFU/strawberry. Rinsing with water reduced populations by 1.24 log. The only treatments capable of inactivating > 3 log of pathogens were (log CFU/strawberry inactivation in parenthesis) 40% ethanol (3.02), and 0.1M sulfuric acid (5.21). Results of other rinses are as follows: 85 ppm paracetic acid (2.84), 0.5% each of lactic and citric acids (2.74), 3% H_2O_2(2.46), 0.33% each of lactic + citric + acetic acids (2.11), 0.1N NaOH (1.84), 200 ppm chlorine (1.73), 30% ethanol (1.59), 1% lactic or 0.5% each of lactic and acetic acids (1.41), 1% acetic acid (1.34), and 0.5% each of acetic and citric acids (1.23).

Significance: *Salmonella* and EHEC inoculated onto strawberries are very resistant to inactivation by sanitizing solutions (as has been demonstrated with other types of fresh produce). Common commercial rinses (e.g., 200 ppm chlorine and 85 ppm paracetic acid) inactivated only 0.5 and 1.6 log more cells than did rinsing with water alone, indicating that more effective commercial sanitizers are needed for inactivating pathogens on strawberries.

T1-12 Sanitizer Efficacy against *Salmonella* during Simulated Commercial Packing of Tomatoes

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Introduction: During tomato packing chemical sanitizers are usually added to the dump tank water to minimize cross-contamination. However, the efficacy of sanitizers continues to be questioned.

Purpose: This study assessed the ability of three commonly used commercial sanitizers to reduce *Salmonella*populations on tomatoes, in wash water and on equipment surfaces using a pilot-scale processing line.

Methods: Five sanitizer treatments - 40 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 40 ppm mixed peracid (Tsunami 200, Ecolab) and 40 ppm free chlorine from XY-12 (Ecolab) at pH 7.8 or pH 6.0 (adjusted with SmartWash (SW) or citric acid (CA)) were evaluated in triplicate for efficacy against *Salmonella* Typhimurium LT2 (avirulent) on 25 lbs (11.3 kg) of dip-inoculated tomatoes (~6 log CFU/g) that were washed for 2 min in a 3.3-m dump tank equipped with two overhead spray jets and then dried on a 0.4-m × 1.5-m polyethylene roller conveyor with sanitizer-free water serving as the control. Two tomatoes (~500 g) and individual water samples (400 ml) were collected at 15-sec intervals during washing with ten dump tank (100 cm^2), four water tank (100 cm^2) and six roller conveyor (350 cm^2) surface samples collected after washing using Kimwipes®. All samples were appropriately neutralized, hand-rubbed/stomached, diluted and surface-plated on trypticase soy agar containing 0.6% yeast extract, 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate with or without membrane filtration to enumerate *Salmonella*.

Results: All sanitizer treatments were more effective ($P < 0.05$) than the water control with XY-12 + CA yielding a significantly greater ($P < 0.05$) reduction for *Salmonella* on tomatoes (3.16 log CFU/g) as compared to Tsunami 100 (2.53 log CFU/g), Tsunami 200 (2.49 log CFU/g),

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XY-12 (2.10 log CFU/g) and XY-12 + SW (1.98 log CFU/g). The same five sanitizers yielded *Salmonella* population of -2.18, 0.62, 0.12, -1.66 and -2.26 log CFU/ml in the dump-tank water after washing. The water control yielded a *Salmonella* transfer rate of 94.1% for the dump tank water and 0.003, 0.002 and 0.001% for surfaces of the dump tank, water tank and roller conveyor after processing with all equipment samples negative for *Salmonella*when sanitizers were used during processing.

Significance: All sanitizers were able to prevent cross-contamination after washing with XY-12 + CA most effective against *Salmonella* on tomatoes. However, additional steps, including acidification of dump tank water containing chlorine-based sanitizers, are still needed to better ensure end product safety.

T2-01 Residues from Chlorine Dioxide Gas Treatment, Generated from Different Delivery Systems, in Fresh Produce

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Developing Scientist Competitor

Introduction: Chlorine dioxide (ClO_2), a strong oxidizing agent, can be applied as a gas in the packaging system acting as antimicrobial. Applications are limited due to concerns regarding residues left on the treated foods' surface, since $ClO_2$is very unstable, breaking down to chlorites and chlorates.

Purpose: This study aimed to determine ClO_2 and its by-products left on fresh produces after being treated with ClO_2gas generated by different systems.

Methods: Cherry tomatoes and fresh-cut Romaine lettuce were exposed to different concentration of ClO_2, i.e., 3, 6 and 10 mg ClO_2/l, for 15-60 min and 1, 3 and 6 mg ClO_2/l for 5-30 min, respectively. The gas was generated by 3 different methods: sachet with dry precursors (z-series, ICA TriNova, Newnan, GA), ClO_2 solution (precursor ICA TriNova, Newnan, GA) and gas generator machine (Minidox-M,ClorDiSys, Lebanon, NJ). Chlorine dioxide and chlorite (ClO_2-) residues were determined by Amperometric titration (4500-ClO_2.C), using an automatic titrator (T50 – Mettler Toledo, Columbus, OH).

Results: Residue on fresh cut lettuces increased with increasing treatment concentration and time independent of the delivery systems, but when treated with the gas generated by the machine more residues were found, e.g., at 6mg/l for 15 min; 23.00 ± 2.69 mg ClO_2/kg and 14.12 ± 3.74 mg ClO_2/kg of ClO_2 and ClO_2- were recovered, when treated with gas from continuous ClO_2 generation machine and sachet respectively. Tomatoes residues were low when treated with the machine or sachets, 0.27 ± 0.22 mg ClO_2/kg, but no residue was found when treated gas generated from the solution.

Significance: Each delivery system has a specific delivery profile that will determine the residue on the fresh produce. The difference in residue found on different fresh produce from each delivery system is significantly different. It is important to identify the ClO_2 delivery system when considering ClO_2 absorption and levels of residues.

T2-02 Modeling of Bisphenol A Migration from LDPE into Food Simulants

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Developing Scientist Competitor

Introduction: In recent years, there is concern about the role of Endocrine Disrupting Compounds (EDCs) in human health. As one of the EDCs, Bisphenol A (BPA) is widely used in food packaging. Migration of BPA from food packaging materials has been investigated.

Purpose: By using mathematical models, this study aims to describe the migration process of BPA from packaging system into food system.

Methods: LDPE films containing 0.1, 0.25 and 0.5 wt % BPA (Sigma-Aldrich, Milwaukee, WI) were prepared by melt mixing followed by compression molding. Reflux extraction was adopted in the determination of initial BPA concentration in LDPE films. Quantification of BPA was carried out by HPLC-UV (Waters, Milford, MA). Migration testing of BPA from LDPE into food simulants was performed according to ASTM D 4754-98, with three factors taken into account: temperature (40, 60 and 80 °C), initial BPA concentration (0.1, 0.25 and 0.5 wt % nominal) and food simulant type (water, 3% acetic acid and ethanol). Fick's diffusion equations were applied to the migration modeling. Diffusion coefficient (D_p) and partition coefficient ($K_{p,F}$), were determined by fitting the migration curve with the diffusion equation.

Results: D_p values obtained under different conditions ranged from 10^{-10} to 10^{-8} cm^2/s. For the interaction effect, only the interaction between temperature and food simulant type was significant ($P < 0.01$). The dependence of D_p on temperature followed an Arrhenius type of relationship. An exponential relationship was found between D_p and initial BPA concentration. Based on the statistical analysis, a relationship can be built to express the diffusion coefficient as a function of temperature and initial BPA concentration for each food simulant. Therefore, D_p values at other temperature and initial BPA concentration can be predicted and the migration level at different time can be calculated from the diffusion equation with those $D_p$values.

Significance: The application of modeling minimizes the experimental work while making accurate predictions.

T2-03 A Comparison of the Effectiveness of Allergen Verification Methods and Test Kits in a Real-Time Food Manufacturing Environment

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Introduction: With increasing global concerns about allergen control and management in the food manufacturing industry, processors are faced with the dilemma of selecting the most suitable method for ongoing verification of their hygiene procedures used to minimise the risk of cross contamination. During the last ten years there have been 425 reported food safety incidences related to unintentional allergen presence in products sold in Europe.

Purpose: To determine the most practical, reliable and cost-effective method of allergen verification in real time food manufacturing environments (Small Medium Enterprises) in line with the new requirements of the Global Standard for Food Safety (Issue 6).

Methods: A total of 5 different tests were used to verify the effectiveness of cleaning to remove allergens (gluten, soya, almonds, milk, celery and egg). The test methods used were visual, surface ATP, total protein (incubated at room temperature), total protein (incubated at 55 °C) and allergen-specific lateral flow. Testing was completed at 5 different food processing sites (bakery, baby food and immune-compromised meals, meat products and ready meals). Testing was completed using the exact instructions provided by the swab/test kit supplier.

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Results: Allergen presence was demonstrated by each test method pre clean and post clean. A total of 200 tests were completed using above methods across various surfaces *in situ*. Post clean 100% of the lateral flow tests were negative (no allergen detected). However, 86.5 % of the 'total protein' tests on the same surfaces were positive indicating protein presence and the ATP test results on some surfaces did not correlate to the total protein test results

Significance: The results identified 'total protein' test as the most sensitive method to assure the manufacturer that allergen cross-contamination had been minimised and therefore reduce the risk of unintentional allergen presence in product and the potential of a product recall. This is especially significant when more than one allergen is present in the manufactured product pre cleaning.

T2-04 Efficacy of Yeast Enriched Either with Glutathione (GSH) or with Selenomethinonine (SE) to Decrease Ochratoxin A Genotoxicity in Human Renal Cells and in Poultry

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Introduction: Ochratoxin A (OTA) a mycotoxin contaminating mainly cereals but also other crops, can be accumulated in meat, and is not destroyed by food processing. It is nephrotoxic for pig and poultry, and is implicated in Balkan endemic nephropathy and associated urothelial cancer in human. One of the most promising and economical strategy for reducing animal exposure to mycotoxins is the utilization of adsorbents in feed to reduce gastrointestinal absorption of mycotoxins

Purpose: The aim of this paper is to evaluate the capacity of several yeasts to decrease genotoxicity of OTA and establish if the decrease is only due to adsorption of OTA on yeast product

Methods: Human renal cells were exposed to OTA (10 μM) alone or in presence of yeast enriched with glutathione (GSH) (10μM) or with selenomethinonine (SE) (10μM). In parallel 10 poultries per group were fed two days with feed including yeast product, and then were fed seven days with feed including yeast product and OTA. Viability of cells was evaluated using MTS test. Genotoxicity was evaluated by detection of DNA-adduct using P32 post labelling method. In addition OTA derivatives formed in human renal cells or in liver and kidney of poultry were analyzed after extraction by HPLC coupled to fluorimetric detection

Results: OTA significantly decreases cell viability (60%; $P < 0.01$) and induces formation of two OTA-DNA-adducts. Adjunction of pure GSH or GSH-yeast partially restores cells viability (70% versus 60%; $P < 0.05$) and avoid DNA adduct formation, explained by conversion of OTA into OTB and 4 OH OTA. Pure SE does not restore viability whereas SE-yeast has antagonistic effect (110% versus 60%; $P < 0.01$). SE and SE-yeast increase OTA-DNA adduct formation correlated to the appearance of new OTA metabolites

Significance: The decrease of OTA toxicity observed with yeast was not only correlated to adsorption but also to biotransformation of OTA which is modulated by yeast. DNA adduct patterns were correlated with OTA derivatives formed in the kidney. GSH-yeast is better to decrease OTA genotoxicity

T2-05 Molecular Epidemiology of *Campylobacter coli* isolated from Conventional and Antimicrobial-free (ABF) Swine and their Environment

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Introduction: *Campylobacter* is one of the leading pathogens causing foodborne illnesses in the US. Epidemiological evidence has indicated that food animals, including pigs, act as reservoirs of *Campylobacter*strains that can infect humans.

Purpose: To determine the clonality or diversity of *Campylobacter coli* isolated from the conventional and antimicrobial free (ABF) production systems at farm, slaughter and environment using multilocus sequence typing (MLST).

Methods: A total of 129 *C. coli* isolates were selected from fecal, environmental and carcass samples of ABF ($N = 71$) and conventional ($N = 58$) production systems. Seven housekeeping genes (*asp, gln, glt, gly, pgm, tkt, unc*) were amplified using PCR and the amplified product was sequenced. Sequence data was analyzed for the determination of allelic profiles and identification of sequence types (STs). Dendrograms and minimum spanning trees were generated to establish the relationships between the genotyped isolates.

Results: We identified 20 and 13 unique STs in the conventional and ABF production systems, respectively. Isolates with similar sequence types were found between the pigs and their environment at farm and slaughter (ABF: 13, $I_A = 0.1308$; Conventional: 20, $I_A = 0.1357$). Higher genotypic diversity was observed among isolates from the conventional swine production systems (ABF: 0.3455 +/- 0.0901; Conventional: 0.3929 +/- 0.0805). Phylogenetic analysis using the unique STs from the two production systems revealed a genotypically diverse population with the presence of *C. coli*isolates sharing a common ancestry.

Significance: MLST Genotyping of *C. coli* isolates from two distinct production systems unveil a weak clonal population and diverse genetic makeup of this species.

T2-06 Occurrence of Food or Waterborne Illness Outbreaks in Africa in 2011

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Introduction: While foodborne illness outbreaks are regularly reported in developed countries, this is not the case in most developing countries. The pathogenic *Escherichia coli* outbreak which occurred in Europe, May 2011, and*Listeria*outbreak in September 2011, in the U.S. were among many that were reported. However, in Africa and other developing countries, similar news is seldom or not even reported. It may therefore be assumed that people in these countries are immune to such diseases, which is unlikely.

Purpose: This work was conducted to find cases of outbreaks that took place in Africa in 2011 and to establish whether food and/or water contamination constitute a major health risk to Africans.

Methods: Different media such as national newspapers, blogs and websites were surveyed. Medical doctors were also consulted for information on cases of gastroenteritis.

Results: The survey showed that waterborne illness outbreaks were rampant in Africa, occurring with varying consequences in 13 African countries in 2011. Records of cases of gastroenteritis in one government hospital in Mpumalanga, South Africa, from January to October 2011, showed an average of 79 cases of gastroenteritis occurred per month out of number of cases (1,700 average) in accident and emergency units, although records did not show whether gastroenteritis cases were caused by contaminated water or food. Cases of food poisoning were however reported in Madagascar, South Africa and Nigeria. The affected foods were fish, an unknown food and moi-moi (from beans), respectively, in the three countries.

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Significance: This survey shows that waterborne illnesses occur more often than foodborne illnesses in Africa. The reason is not far-fetched. There is lack of good water supply, adequate sanitation and good drainage system which cause water pollution. Food pathogens may not be a serious problem at the moment because foods, including vegetables, are normally cooked for a long period of time and some also are fermented. However, as Africans begin to realize the importance of eating fresh and minimally processed food, there may be more increase in foodborne illnesses cases.

T2-07 Pathogen-annotated Tracking Resource Network for *Vibrio* Risk Assessment and Management

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Introduction: Reported foodborne vibriosis in U.S. has increased since 2000 with the majority of cases attributed to consumption of raw oysters, even though more strict time-to-temperature requirements have been adopted.

Purpose: We report the development of a multi-layered vibrio database in Pathogen-Annotated Tracking Resource Network (PATRN) that can be queried for parameters of interest.

Methods: Data sets on vibrio levels in oysters at harvest thru consumption were obtained from publications and WA and TX state monitoring plans. Illness data was extracted from state and federal databases. Publically available information for multi-locus sequence typing (MLST) of vibrio strains, climate and oyster landings were also incorporated. All data were uploaded to PATRN and data with known locations were formatted into GIS to illustrate geographical distributions. PATRN's existing capabilities were expanded to integrate these data and incorporate query-specific graphics functions for investigating trends and potential impact of variables on illnesses and risk.

Results: PATRN provides a user-friendly format to import and integrate available data for individual queries and graphical illustrations. Analyses can be updated in "real time" as new information becomes available. The data can be segregated to investigate the effects of air and water temperature or salinity on vibrio levels at harvest. The influence of different time-to-temperature regimens or changes in landings on reported illnesses can also be evaluated. Maps illustrating harvest sites linked to illnesses can be generated. Vibrio levels from monitoring data, or environmental factors such as temperature or salinity, can be linked to implicated harvest sites. Additionally, the geographical distribution of strains with specific virulence traits, serotype, and/or MLST type can also be visualized.

Significance: This study demonstrates that the web-based PATRN vibrio data base (*http://www.patrn.net/patrn_contact.html*) can be a powerful tool for evaluation of factors contributing to vibrio risk allowing for timely and targeted risk management.

T2-08 Traceback and Environmental Investigation of an Outbreak of *Salmonella* Enteritidis Associated with Organic Eggs

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Introduction: The Minnesota Department of Health (MDH) and Minnesota Department of Agriculture (MDA) investigated an outbreak of *Salmonella* Enteritidis infections associated with consuming in-shell organic eggs. A traceback investigation linked the cases to an egg facility in Minnesota. An environmental sampling investigation at the facility found the outbreak strain of *Salmonella* Enteritidis at two locations on an egg belt in the laying barn.

Purpose: This outbreak investigation demonstrates the effective use of traceback and environmental investigations to confirm the source of a *Salmonella*Enteritidis outbreak associated with a free-range egg facility.

Methods: MDH epidemiologists interviewed all *Salmonella* Enteritidis cases that matched by pulsed-field gel electrophoresis (PFGE). Eight individuals infected with the outbreak strain of *Salmonella*Enteritidis were identified in Minnesota with illness onsets in August and September 2011. Consumption of organic eggs from a common farm was statistically implicated in a case-control study. Five cases had specific egg purchase or consumption information that MDA investigators used to trace the eggs back to a common farm.

Results: The five cases of illness traced by MDA had purchased eggs from six different retail locations. All six retail locations received product directly from one egg laying facility in Minnesota. An on-farm traceback investigation found the farm used eggs from its own hens and eggs from nine other laying facilities in Minnesota to fill orders. A review of the production records indicated that only eggs from the implicated farm were associated case exposures. Twenty-four environmental samples were collected from the laying barn and candling room. Two samples collected from the egg belt were positive for the outbreak strain.

Significance: With the impending implementation of the FDA's Egg Rule for small egg producers, this outbreak highlights the challenges associated with free-range organic egg production and some potential issues for producers in complying with the new regulations.

T2-09 Reactive Arthritis Incident Estimates from Four Foodborne Pathogens

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Introduction: Reactive Arthritis (ReA) is an inflammatory arthritis caused by certain Gram-negative foodborne and venereal infections. While foodborne illness associated ReA affects males and females with the same frequency, the postvenereal form occurs at a male to female ratio of 9:1. These bacteria, or their DNA components, have been found in the synovial tissue or fluid of persons suffering with ReA. Gastrointestinal infections, even minor, precede arthritis symptoms usually by 1 – 6 weeks.

Purpose: The incidence of ReA post-enteric infection is not well understood or defined, the overall attack rate of foodborne illness associated ReA ranges from 1.5% to 30%. Lack of a clear disease definition and specific diagnostic criteria for ReA makes epidemiologic studies problematic.

Methods: This analysis looked at published studies of ReA for four bacterial foodborne infections. *Shigella, Yersinia, Salmonella,* and *Campylobacter*incident rates are estimated for 2010 through data collected by FoodNet sites. Incidence of ReA for 2010 are approximated using: census estimates for 2010, FoodNet incident rates of illness, and studied outcomes of specific cohorts infected with those 4 bacterial infections.

Results: Results approximate that ReA is a public health burden of significant consequence affecting as few as 4,616 individuals annually in the United States or upwards of 19,257. Given that ReA can affect 50% of cases chronically, 12,000 people may annually contract the chronic form. This is a conservative estimate, since some studies suggest 63% will develop chronic complications of ReA.

Significance: Increased recognition of the severity and incidence of ReA following foodborne infection, may prompt improved treatment of the initial infection with antibiotics, thus dramatically alter the subsequent attack rate of ReA.

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T2-10 Foodborne Disease Outbreaks Attributed to Peanuts, Tree Nuts and Association Products, United States 1998-2009

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Introduction: Several large outbreaks associated with contaminated peanut butter have occurred recently in the United States. We summarized recent outbreaks associated with nuts, including peanuts and treenuts, and associated products.

Purpose: To describe the frequency and characteristics of outbreaks attributed to nuts and associated products.

Methods: The Centers for Disease Control and Prevention (CDC) conducts surveillance for foodborne disease outbreaks. We reviewed data for outbreaks in which nuts or a product made from nuts was reported as the food vehicle during 1998- 2009. We analyzed outbreak frequency and size, hospitalizations and deaths, pathogens, and settings of food preparation.

Results: From 1998-2009, 11 outbreaks were reported, resulting in 1,645 illnesses, 309 hospitalizations, and 10 deaths. At least one outbreak occurred in each reporting year except 1999, 2000, and 2009. Three outbreaks were associated with peanuts, three with almonds, two with peanut butter/peanut paste, two with unspecified nuts, and one with cashews. The average outbreak size was 20 illnesses; the two peanut butter outbreaks were particularly large and severe, sickening more than 700 persons each and accounting for all but one of the deaths. Of the eight outbreaks with an etiology reported, five were caused by *Salmonella* (serotypes Enteritidis [2 outbreaks], Tennessee, Thompson, Typhimurium), two were caused by chemicals, and one was caused by norovirus. In five outbreaks the etiologic agent was isolated from the implicated food. Six outbreaks were associated with nuts distributed as a commercially pre-packaged product (peanut butter [2 outbreaks], peanuts [2 outbreaks], almonds, cashews); three involved products distributed to and causing illness in multiple states.

Significance: Outbreaks associated with consumption of contaminated nuts or products made from nuts occurred regularly in recent years. Most involved commercially pre-packaged products. Because nuts are frequently consumed raw, these findings underscore the importance of on-farm and processing practices to ensure food safety.

T2-11 Integrating Information from Outbreaks, Expert Elicitation, and Case-control Studies to Attribute Foodborne Illness to Foods

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Introduction: Attribution of foodborne disease to food sources is critical for resource allocation and for developing and evaluating food safety interventions. Numerous attribution approaches have been developed, each with distinct strengths and weaknesses, but few attempts have been made to combine information from multiple data sources into integrated estimates.

Purpose: This study's objective is to assess the reliability of multiple sources of food attribution information – namely foodborne outbreak data, expert elicitation, and case-control studies – and to use these results to create integrated attribution estimates for 14 major pathogens.

Methods: This study builds on prior work evaluating outbreak attribution based on multiple reliability measures: outbreak density, ratio of estimated incidence to reported outbreak cases, sum of mean differences squared between outbreak and expert attribution, and the mean standard deviation across experts. We develop a novel approach to integrating attribution data from multiple sources that uses expert elicitation results to weight primary sources.

Results: Outbreak attribution for *Campylobacter*, *Toxoplasma*, *Cryptosporidium*, and *Yersinia* are shown to be unreliable based on multiple metrics, while estimates for *E. coli* O157:H7, *Vibrio* spp., and *Cyclospora* are the most reliable. The mean standard deviation in expert results is highest for *Toxoplasma* (2.02) and norovirus (1.77) suggesting the need for improved attribution for these pathogens. We present previously unpublished integrated attribution estimates for 14 pathogens over 12 food categories.

Significance: Outbreak data is shown to have variable reliability across pathogens as a source of attribution information, while expert elicitation is shown to be a powerful tool for evaluating attribution from multiple data sources when designed for such comparisons. Our findings suggest that creating combined estimates of attribution of illnesses to foods are possible and may be more reliable for public health policy than estimates based on a single data source.

T2-12 Coagulase-negative Staphylococci (CoNS): Reservoir of Multidrug Resistance in Animals

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Introduction: Coagulase-negative staphylococci (CoNS) in animals are important reservoir of antimicrobial resistance; however, there are limited studies on the susceptibilities of CoNS to non-β-lactam antimicrobials.

Purpose: This study aims to understand the susceptibilities of CoNS to non-β-lactam antimicrobials and identify the resistance determinants.

Methods: A total of 87 CoNS recovered from food animals were characterized by antimicrobial susceptibility testing, resistance gene identification, and conjugation tests.

Results: Of 7 staphylococcal species studied, *S. lentus*, *S. sciuri*, *S. xylosus*, and *S. haemolyticus*, accounted for over 96% of the isolates. In addition to β-lactam resistance, high percentages of CoNS were resistant to tetracycline (67.8%), erythromycin (36.7%), clindamycin (27.5%), and quinopristin/dalfopristin (14.9%). A total of 47 isolates (54%) were resistant to at least 3 antimicrobial classes, including 6 CoNS resistant to 6 antimicrobial classes. The common genes for the above mentioned resistance phenotypes were *mec*(A), *tet*(M), *erm*(A), and *vga*(A), which were identified from 68.7%, 61%, 56.2%, and 69.2% of the isolates, respectively. *tet*(M) was conjugatively transferable from 11 tetracycline-resistant CoNS to a*Enterococcus* strain, underlining the potential of antimicrobial resistance transfer from *Staphylococcus* to the commensal bacteria in human.

Significance: This study suggests that multidrug resistance is common in CoNS in animals and adds to our knowledge of the potential of CoNS serving as antimicrobial resistance donors in the food production environment.

T3-01 Factors That Predict the Likelihood of *Listeria monocytogenes* Contamination in Produce Fields

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Introduction: *Listeria monocytogenes*has been associated with produce-borne outbreaks in cabbage, celery, and melons.

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Purpose: A longitudinal study was conducted, using five produce farms in New York State, to characterize prevalence, persistence, and diversity and to determine landscape and meteorological factors that predict the likelihood of *L. monocytogenes* in the preharvest environment.

Methods: Over the two-year study, farms were sampled four times a year and 588 samples were collected. Soil, water, fecal, and drag swab samples were collected from four fields per farm on each visit; GPS coordinates for each site were recorded and revisited. Samples were cultured for *L. monocytogenes*; isolates were confirmed by PCR, and sequenced in the *sigB*gene.

Results: The prevalence for *L. monocytogenes* was 15% (88/588). Farm, season and sample type were found to be significantly associated with the frequency of *L. monocytogenes* positive samples (P = 0.0008, 0.0010, 0.0001). The frequency of *L. monocytogenes*-positive samples was highest amongst water samples (48/174). Analysis of *sigB* sequences showed a high diversity of *L. monocytogenes* allelic types amongst farms (D = 0.80 ± 0.10). There was evidence of persistence on one farm, demonstrated by the recovery of *L. monocytogenes* isolates with the same allelic type at least three times from the same location. Sample sites were partitioned into high and low prevalence clusters according to classification tree (CT) models based on landscape and meteorological data. Proximity to water (> 37.5m) was identified as the most important factor influencing the likelihood of a *L. monocytogenes*positive sample, but temperature, soil properties, and proximity to livestock production and urban development were also important.

Significance: This information can be used to develop risk maps that indicate locations where the prevalence of *L. monocytogenes* is higher, which consequently may pose a greater risk for contamination of produce. These findings will support the development of science-based mitigation strategies for growers.

T3-02 Development of an Interactive Modeling Tool to Predict the Risks Associated with Contaminated Fresh-cut Lettuce in Canadian Distribution Systems

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Introduction: The assessment of risks associated with the contamination of fresh-cut lettuce with pathogens such as *Escherichia coli* O157:H7 would benefit from the means to map the spatial distribution of contaminated product over time and predict potential public health impacts.

Purpose: The objective of this work was to develop a modeling tool capable of 1) mapping the distribution of contaminated fresh-cut lettuce from processing to retail; 2) predicting pathogen levels in packaged lettuce; and 3) estimating consumer accessibility and risk over time.

Methods: A relational database was constructed to store relevant data including: geographical coordinates of Canadian processors, distribution centres, retail outlets; product volumes, time and product temperature at each stage; transportation routes and census data. ArcGIS™ Tools (Network Analyst, Spatial Analyst) were used to calculate transit times and to quantify population accessibility for each retail outlet. The overall risk model incorporating a predictive equation for *E. coli* O157:H7 in packaged lettuce was designed with Arena™ software. Visual Basic (VA) was used to develop an interface to dynamically display the distribution of contaminated product and the predicted risk index.

Results: The performance of the tool was demonstrated for a scenario whereby 3600 kg of fresh-cut lettuce contaminated with 1 x 10^3 CFU/g *E. coli* 0157:H7 were processed in one plant that supplies two retail chains in one Canadian province. Product distribution and populations most at risk of purchasing contaminated product were measured over time and results were mapped. The model predicted a twelve-day outbreak due to contamination in 3888 of 710,978 retail packages sold in the province. The average prevalence of contaminated packages in implicated retail outlets varied between 9.8 to 14.5%, depending on day.

Significance: The ability to model the spread and impact of contaminated products will enable more accurate estimates of food safety risks and improved identification of vulnerabilities in fresh-cut produce distribution systems.

T3-03 Risk Assessment of Field Survival of *Salmonella enterica* and *Escherichia coli* O157:H7 Surrogates on Cilantro in Relation to Sequential Cutting, Re-growth and Postharvest Washing and Storage

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Introduction: Since 2004, the FDA has confirmed the presence of *Salmonella* species and Shiga toxin-producing *Escherichia coli* in 28 fresh cilantro samples in or entering marketing channels from both domestic and non-US origin. Cilantro is a widely enjoyed culinary herb commonly consumed in its raw state without a terminal kill step.

Purpose: To assess the comparative post-contamination consequences on cilantro, in model systems, with attenuated isolates of *S. enterica* and *E. coli* O157:H7 during open-field production, sequential harvest and crop re-growth, and in simulated postharvest washing.

Methods: Two cultivars of cilantro, Santo and Leisure, were grown under open field conditions and spray-inoculated with two inoculum doses (log 4 and log 6 CFU/ml) of attenuated strains of *E. coli* O157:H7 and *S. enterica*sv. Typhimurium. Cilantro was harvested at commercial maturity stage, processed in a model wash system amended with NaClO (50 mg/l, pH 6.5) and stored for 14 days at 5 °C or 12.5 °C. Quantitative and qualitative survival of the pathogens was monitored after 12 h, 6 and 12 days post inoculation (dpi), following wash processing, and 7 and 14 days post washing. The effect of delays to cooling after harvest and the persistence of the pathogens in sequential cuttings and re-growth intervals was also evaluated.

Results: No significant variability of attached *E. coli* O157:H7 and *Salmonella* was observed as a function of cultivar. *Salmonella* and *E. coli*O157:H7 populations declined after inoculation below the limit of quantitative detection, but viability was demonstrated after 12 dpi following selective enrichment. Washing with 50 mg/l of NaClO was not sufficient to disinfect the inoculated cilantro (log 6) prior to refrigerated storage. Viable populations of both pathogens were confirmed throughout the storage interval. No applied bacteria were detected 22 days after re-growth of cilantro plants in the field.

Significance: This study provides preliminary risk-based data that will be useful for the development and adoption of preventive controls in food safety management among cilantro growers and processors.

T3-04 Risk Assessment and Spatial Distribution of Human Pathogen Contamination of a Cantaloupe Field Adjacent to a Small Dairy Operation

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Introduction: Adjacent land use and proximity to point and non-point sources of contamination impact the microbiological safety of fresh produce. Well recognized sources include irrigation water, accumulated animal waste, soil amendments, and proximity to an Animal Feeding Operation.

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Purpose: To perform an assessment of prevalence and spatial distribution, including the potential risk, based on indicators and molecular evidence, and confirmed presence of *Escherichia coli* O157:H7, including other Shiga toxin-positive*E. coli,* and *Salmonella enterica*, in a cantaloupe field located, at one edge, in immediate proximity to a dairy.

Methods: Evaluation of total coliforms, fecal indicators and detection of molecular markers for human pathogens was performed approximately 2 weeks before the estimated harvest date, at commercial maturity, and after field discing on fruit, soil and environmental samples including irrigation water, manure, and bedding.

Results: Quantification of total coliforms on melons provided evidence of greater populations in areas adjacent to animal corrals that corresponded with elevated soil organic matter and nitrogen content. Detection of the fecal indicators*Enterococcus* and *E. coli* was encountered in diverse environmental samples across substantial spatial distances and all time points. Molecular markers for *E. coli* O157:H7 and pathogenic *E. coli*, particularly *eae* and *stx*2 genes were observed on multiple dates. *E. coli* O157:H7 ($stx1^-$) was isolated and confirmed from fruit and soil. Detection of pathogenic *E. coli* and *Salmonella* markers was positive on the harvest-decision date and the crop destroyed. After discing of the field, total coliforms and *E. coli*increased 2-3 log CFU/100 g along the entire field. Detection of pathogenic *E. coli* markers (*eae*A and *stx*2) was still positive and broadly distributed. Following planting, growth and incorporation of a monocot cover crop the *E. coli*populations and pathogen virulence markers were no longer detectable.

Significance: Proximity to animal operations can significantly alter the microbiological quality of adjacent crops and variable factors must be evaluated to establish a scientific basis for recommended buffer setbacks in produce guidance and audit schemes.

T3-05 Risk Factors for Microbial Contamination in Fruits and Vegetables at the Pre-harvest Level: A Systematic Review

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Introduction: Microorganism contamination is known as the main cause of foodborne illness related to fresh produce. *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 are among the most important pathogens of concern to food safety.

Purpose: The objective of this study was to perform a systematic review of risk factors for contamination of fruits and vegetables with *L. monocytogenes*, *Salmonella*, and *E. coli*O157:H7 at the pre-harvest level.

Methods: Relevant studies were identified by searching 6 electronic databases: MEDLINE, EMBASE, CAB Abstracts, AGRIS, AGRICOLA, and FSTA. Searches were conducted using the following thesaurus terms: *L. monocytogenes*, *Salmonella*,*E. coli*O157 AND fruit, vegetable. All searching terms were exploded to find all related subheadings. To be eligible, studies had to be prospective controlled trials or observational studies at the pre-harvest level and had to show clear and sufficient information on the process in which the produce was contaminated.

Results: Out of 3,463 identified citations, 68 studies fulfilled the eligibility criteria. Most of these studies were on the leafy greens and tomato. While many risk factors for the pre-harvest level produce contamination have been evaluated, the quality assessment of the reviewed studies confirmed existence of solid evidence for only some of them, including produce growing on the clay-type soil, application of contaminated or non-pH-stabilized manure and usage of spray irrigation with contaminated water, with a particular risk of contamination on the lower leaf surface. Weak or missing evidence of association with produce contamination was determined for additional risk factors, including for the effect of animal host's diet, wild animal intrusion and floods.

Significance: Synthesis of the reviewed studies suggests that reducing microbial contamination of irrigation water and soil are the most promising targets for prevention and control of produce contamination. Furthermore, this review provides an inventory of the evaluated risk factors, including those requiring more research.

T3-06 Cross-contamination and Distribution of *Salmonella* in Processed Fresh Apples

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Introduction: Pathogens can be present in fruits and vegetables because of contamination events occurred in the field or during processing (cross contamination or recontamination). However, due to both the low pathogens incidence and sampling limitations, little is known about how bacteria are distributed or which concentration and prevalence levels are given in the final product.

Purpose: To study the effect of cross contamination during processing on the spread of *Salmonella* in processed fresh apples.

Methods: A commercial process of packaged fresh apples was simulated at lab-scale. To simulate a natural contamination, one apple was spot-inoculated with *Salmonella* and introduced into a lot of 30 apples which were consequently processed according to the following steps: overnight storage (4 °C), washing, pre-dry on sponges, air-dry and packaging in plastic bag. After processing, all processed apples, sponges and wash water were sampled and analyzed for *Salmonella*. The experiments were carried out using two inoculums levels in the initially contaminated apples: high (8 log CFU/apple) and low (5 log CFU/apple) level.

Results: *Salmonella* counts after the storage step at 4 °C dropped >2 log CFU/apple. At high inoculum level, all processed apples, sponges and wash water were positive for *Salmonella*. At low contamination level, only the initially inoculated sample was positive while the rest of processed apples were found negative after a standard enrichment method. Even though no cross contamination occurred at low inoculums level, *Salmonella* was found in wash water (1/2) and sponges (3/4). Based on generated data, a stochastic model was derived to be used to validate and optimize sampling plans.

Significance: This information can be relevant to establish and develop effective sampling plans and food safety criteria.

T3-07 An Integrated, Risk-informed System for Informing Food Safety Decision Making

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Introduction: A risk-informed system is integral to a decision-making framework that will address public health. With the visibility of the President's Food Safety Working group and the new authorities in the Food Safety Modernization Act, there are unprecedented opportunities to move forward with systematic science-based, risk-informed strategies for food safety decision making. The lack of a systematic approach to decision making can result in unwanted outcomes, from a decrease in public trust to unintended consequences in the marketplace, the environment, society, and the political realm.

Purpose: The purpose of this project was to develop a "proof-of-concept" Integrated Decision Support System (iDeSSy) for food safety risk management. Such a system must be data driven and public health focused, but it must also consider other significant components beyond public

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health including economic considerations, societal factors, and public perception, among others.

Methods: iDeSSy includes a central relational database housing data on hazards, food commodities, and consumers and four individual modules for (1) ranking hazard-commodity pairs based on risk factors related to public health; (2) prioritizing hazard-commodity pairs based on decision criteria other than public health; (3) evaluating mitigation options with respect to their costs and impacts on risk; and (4) optimizing the process of resource allocation among available mitigation options.

Results: We evaluated iDeSSy using a series of case studies to demonstrate that the system can enable decision makers to evaluate the food safety system in a comprehensive way and to follow a systematic process for evaluating food safety problems.

Significance: iDeSSy offers a number of features that are critical in developing a decision support system including data-driven results, stakeholder involvement, transparency, flexibility, availability of multiple decision criteria, support for scenario evaluation, and optimized allocation of available resources.

T3-08 Foodborne Contamination Consequence Modeling

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Introduction: Intentional foodborne contaminations have been attempted in the United States and abroad. Several online tools, software packages, and approaches have been developed and are available for evaluating potential vulnerabilities within the food supply chain. However, there remains a need for robust, risk-based consideration of various threats to the U.S. food supply (from 'farm-to-fork').

Purpose: As part of the Chemical Terrorism Risk Assessment (CTRA), a DHS CSAC funded program, Battelle has developed a foodborne contamination consequence model that estimates human health consequences of various intentional food contamination scenarios.

Methods: Knowledge from published literature and subject matter expertise on agent stability, food processing technologies, and supply chain vulnerabilities were applied to evaluate the impact of chemical threats on public health. Foodborne contamination scenarios were mathematically simulated from the point of contamination (e.g., a storage silo), through food processing (e.g., pasteurization), packaging or bottling, distribution to retail or quick service restaurant (QSR) outlets, to points of sales of contaminated product, consumer and QSR preparation methods (e.g., cooking), and consumption patterns. A recall or public announcement component considered the time to the appearance of illnesses or injuries, and applied a rate of information diffusion to calculate the amount of contaminated product removed from retail outlets and consumer homes.

Results: Contaminant-specific data, such as that for dose-response, hydrolysis rate, temperature-dependent decay, and time to symptom onset, were utilized to provide estimates of potential consequences in terms of injuries of varying severity. Results were generated, illustrating the effect of various contamination points and mitigation steps on public health consequences.

Significance: These modeling capabilities can be applied to prioritize investments in mitigating a food contamination event and have other potential applications such as determining optimal, risk-based contaminant sampling schemes for naturally occurring or accidental contamination events.

T3-09 Development of a Web-based Tool for Assessing and Managing Microbial Risk in Minimally Processed Vegetables and Ready-to-Eat Meat Products

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Introduction: Ready-to-eat meat products and minimally-processed vegetables are of growing importance in Europe as much as it is in the US. These types of food should be among the most relevant in order to be submitted to risk assessment, as they are widely spread, and its safety could be compromised, since scientific and epidemiologic evidence has shown that this type of foods can be contaminated by pathogens coming from primary production or the factory environment (cross-contamination or recontamination).

Purpose: The aim of this project was to develop a computer tool, easy to use by risk assessors and managers, to estimate and mitigate microbial risk in minimally-processed vegetables and ready-to-eat meat products.

Methods: The methodology used to develop the web-based tool was based on the risk assessment framework and comprised 6 phases: 1) hazard identification; 2) modeling processes along farm-to-table chain with a flexible structure by means of predictive and/or stochastic models; 3) characterization of identified hazards; 4) development of a model for risk characterization; 5) proposal and implementation of risk management measures; and 6) development of a computer tool involving the results of the previous objectives, and easy to use and interpret. For vegetables, the development was greatly benefited by the experience gained through a US research project. ("Systems Approach to Minimize *Escherichia coli* O157:H7 Food Safety Hazards") in which different mitigation strategies were studied for *E. coli* O157:H7.

Results: The design of the tool was based on an object-oriented approach, which enables to design specific food processes by combining 4 basic events: bacterial transfer, survival, growth, and inactivation. With this tool, assessors and managers are able to introduce data which are usually confidential (e.g., prevalence and concentration of pathogens in foods from official analyses), and obtain a final estimate of the risk. Besides this, it is also possible to select different management measures by applying a scenario analysis and to know their impact on risk mitigation.

Significance: The realization of this project represents a decisive advance in the assessment and management of safety of these type of foods in Europe and the US, demonstrating transparency with scientific basis in making decisions.

T3-10 Probabilistic Dietary and Microbial Risk Assessment Software

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Introduction: Accurately quantifying microbial risk in a population of consumers requires modelling both microbial growth and dietary exposure. The factors that influence both elements are intrinsically variable and can require millions of simulations in order to sufficiently estimate the distributions of pathogen concentrations, exposure, and dose-response. Rapid risk assessment and scenario analysis can require considerable time and computational effort as a result.

Purpose: To develop high-performance microbial risk assessment software, incorporating variable environmental conditions in the supply chain and actual consumer consumption patterns.

Methods: Predictive models were implemented stochastically for a number of pathogen/commodity pairs to simulate all potential variability at various points in the supply chain (e.g., variable initial conditions, storage times, temperatures, pH, etc.). Extensive experimental data from the ComBase database was used to estimate growth parameters. The calculated distribution of pathogen concentrations in a number of commodities was combined with probabilistic dietary exposure and dose-response models in order to estimate the disease incidence in a population of consumers, using national consumption surveys from the EU and the US (NHANES). The power of cloud computing was used in order to handle the large data sets and computational effort required.

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Results: The method was successfully applied to a number of scenarios, including *E. coli* O157:H7 in beef and *Salmonella* Typhimurium in chocolate. An assessment involving 100,000 simulated consumers using the US NHANES survey, 40 food beef commodities contaminated with varying levels of *E. coli*O157:H7, under varying multi-stage environmental conditions, can be completed in less than 30 minutes. Complete output for a given assessment was stratified to determine e.g., the drivers of pathogen growth, consumer exposure, vulnerable subpopulations, etc., enabling appropriate risk mitigation strategies to be identified.

Significance: This presents a valuable tool that can be used for routine risk assessment and rapid post-outbreak analysis. The tool was developed for the food industry, regulatory bodies, and academic research in microbial food safety, and is extensible to a broad number of pathogens, commodities, and consumer populations.

T3-11 The Development and Elaboration of a Risk-based Sampling Plan to Control *Listeria monocytogenes* in a Hospital Food Service Operation

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Introduction: Although listeriosis can occur in healthy adults and children, the most commonly affected populations include pregnant women, neonates, the elderly, and those persons who are immunosuppressed by medications or illness. The high prevalence of *L. monocytogenes* in foods in general, together with a high fatality rate of listeriosis suggests that *L. monocytogenes*represents an important hazard to human health.

Purpose: The occurrence, spread, growth and survival of *L. monocytogenes* in foods and food environments has to be controlled. Therefore, a risk-based sampling plan for the verification of the Food Safety Management System (FSMS) towards *L. monocytogenes*was developed and performed for a hospital Food Service Operation (FSO).

Methods: First the classification of all used food types in various food categories, with similar microbiological ecology towards *L. monocytogenes* took place. Next a score system based on i) the reported epidemiological association of the food type with listeriosis outbreaks, ii) the reported prevalence of *L. monocytogenes* in the food types, and iii) the potential of *L. monocytogenes* to grow or survive during storage and/or further processing, was set up and through summation of the attributed scores, a minimum score of zero (low risk) and a maximum score of nine (high risk) could be obtained. A similar methodology was applied to identify critical sampling locations in the environment where *L. monocytogenes*is possibly present.

Results: The attribution of risks to the defined food categories and critical sampling locations was the basis for selection of food types and environmental samples to be taken up in the sampling plan. Seven food products (n=49) and none of the environmental samples (n=145) were found to be positive for *L. monocytogenes*.

Significance: The developed sampling plan can be used to improve the food safety output of the current FSMS, *i.e.*, to control *L. monocytogenes*, in the FSO.

T3-12 Quantitative Risk Assessment for Campylobacteriosis in New Zealand by the Bayesian Belief Network Approach

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

Purpose: The purpose of this study was to conduct QMRA by Bayesian Belief Network approach which has many advantages.

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

Results: The QMRA indicated that hygiene has a significant impact on the total probability of illness. An increase in the poor hygiene percentage by approximately 50% reflected an increase of approximately 50% in the probability of developing illness; the impact of increasing the contamination prevalence on farm and after plant processing was similar to the hygiene impact. However, the estimated probability of contracting campylobacteriosis by consuming poultry predicted 2000 more cases than the actual notifiednumber from all the sources. This is a more plausible estimate than the QMRA estimate by Monte Carlo method, given the number of unreported cases and the number of campylobacteriosis cases acquired by sources other than poultry consumption.

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

T4-01 Norovirus Survival on Spinach during Pre-harvest Growth

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Introduction: Produce can become contaminated with viral pathogens in the field through soil, feces, or water used for irrigation, through application of manure, biosolids, pesticides, and fertilizers, and through dust, insects, and animals.

Purpose: The objective of this study was to assess the survival and stability of human noroviruses and surrogates including murine norovirus (MNV) and Tulane virus (TV), on foliar surfaces of spinach plants in preharvest growth conditions.

Methods: Mature spinach plants were spray inoculated with human norovirus of both genogroups I and II (NoV GI or NoV G2), MNV and TV (approximately 4 log genomic copies) using an airbrush to deposit virus onto the entire foliar surface of the plant including the stem, adaxial and abaxial surfaces of the leaves. Spinach plants were grown in a biocontrol chamber at optimal conditions of 18 °C, 65% humidity and a light intensity of 150 μmol/m^{2}s for a photoperiod of 15 h and 9 h dark period for up to 21 days. Samples were analyzed for virus presence on days 0, 1, 3, 5, 7, 14 and 21 by qPCR for all viruses and plaque assay for MNV and TV. Controls included virus inoculated HBSS and non-inoculated spinach plants.

Results: MNV and TV were infectious through day 14 at titers of 2.89 ± 0.36 and 2.77 ± 0.15 log PFU, respectively, but were not detected after 21 days on leaves. NoV G2 was detected by qPCR on leaves through day 14 at a titer of 2.91 ± 0.70 log genomic copies, and virus was not detected on day 21. MNV and NoV titers decreased significantly by day 3 ($P \leq 0.05$), but titers did not decrease significantly between days 3, 5, 7

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and 14 ($P > 0.05$). Control plants remained negative.

Significance: These results suggest noroviruses can survive for up to two weeks on foliar surfaces of spinach in environmental conditions. Norovirus surrogates, MNV and TV, showed similar survival in this study to human noroviruses. This understanding of the behavior of enteric viruses on spinach leaves can be used to develop produce growers guidelines and to assess risk under certain growing conditions.

T4-02 Evaluation of Good Agricultural Practices on Minnesota Vegetable Growing Operations

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Introduction: Good Agricultural practices (GAPs) and other hygienic practices (GMP/GHP) are believed to reduce fecal contamination on vegetable products. To date, little work has been done to evaluate GAPs of small-acreage vegetable growers.

Purpose: To document the occurrence of pathogens and fecal indicator organisms on farms that incorporated GAPs versus those that did not. Fifteen farms were selected based on their level of GAPs adherence and compliance [GAPs compliant, n= 8 (GAP A) and GAPs non-compliant, n= 7 (GAP B)].

Methods: Farms enrolled were a mixture of conventional, non-certified organic and certified organic. Each farm was visited 3 times over the growing season. Producers were interviewed with a standard questionnaire and samples were taken from compost, harvesting tools, containers and water samples. Samples were tested for coliforms, *Escherichia coli*, *E. coli* O157:H7, and *Salmonella*.

Results: All enrolled farms grew leafy greens. The average size was 3.5 acres with a median of 5 workers. No seasonal differences in total coliform counts were observed among all the surveyed farms (p=0.13). Water samples from GAP A farms had lower coliform counts (2.5 coliforms /l) compared to GAP B farms (12.56 coliforms /l). GAP B farms consistently had compost containing average coliform counts greater than 10,000 CFU/g compared to GAP A farms (R.R 1.4 95% CI 1.12-1.92, $P < 0.01$). Six (42%) of 14 farms had *E. coli* isolated from compost samples. Two had levels of *E. coli* with more than 100 CFU/g. There was no difference in recovery of *E. coli* between GAP B (26.1%) and GAP A farms (22.2%). *Salmonella*was not recovered from participating farms.

Significance: We observed differences in coliform counts from compost piles between farms that incorporated GAPs vs. those that did not. Additional work is needed to evaluate the effect of GAP incorporation on product safety.

T4-03 Identification of On-farm Bacterial Reservoirs and Potential Contamination Routes for In-field Leafy Greens

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Introduction: Although contamination of produce may occur throughout the food continuum, focus is primarily directed at minimizing in-field and harvest-based contamination. In British Columbia (BC), Canada, data identifying microbiological risk in produce are lacking, and consequently hampers development of BC-specific on-farm food safety programs.

Purpose: Identify microbiological reservoirs and dissemination routes contributing to the microbiological quality of in-field leafy greens.

Methods: Pre-harvest plant (n=423) and environmental samples (irrigation water, harvesting equipment, hand swabs, soil; n=107) were collected from an organic production system in BC weekly between August and October 2011. Levels of coliforms and *Escherichia coli* were determined using 3M Petri-Films. *Enterococcus* spp. were detected using Enterococcosel enrichment broth and agar. Recovered *E. coli*isolates were subjected to antibiogram and multiplex PCR–based phylogenetic typing.

Results: Coliform levels on in-field leafy greens ranged from 0.7 to 1.8 log CFU/g (average; 1.1 ± 0.1 log CFU/g). The overall prevalence of *E. coli* recovered from leafy greens was 1.7%. Generic *E. coli* were recovered repeatedly from the irrigation water reservoir (32%), overhead sprinkler (67%), soil (43%), and worker hand swabs (15%). *E. coli*isolates belonging to all four phylogroups (A, B1, B2, D) were detected in the irrigation reservoir well, whilst only B1 isolates were recovered from the sprinkler. Workers' hands showed the presence of isolates belonging to A and D, and soil samples revealed the presence of B1 and D phylogroups. Common to isolates recovered from identified reservoirs and isolates on leafy greens were phylogroups A, B1 and B2, suggesting possible dissemination.

Significance: Overall, although numerous *E. coli* reservoirs were identified on-farm, levels of coliforms and *E. coli* recovered from in-field leafy greens were low. Despite this, phylogrouping data highlight possible transmission routes and the consequent need to develop intervention strategies that disconnect potential contamination routes from the farm environment to produce.

T4-04 *Escherichia coli* O157:H7 in the Spinach Pre-harvest to Post-harvest Continuum: Implications for Preventive Control Programs

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Introduction: Understanding the dynamics of *Escherichia coli* O157:H7 (*Ec*O157) behavior within the pre-harvest to post-harvest continuum of spinach would improve preventive control programs.

Purpose: To evaluate the survival, prevalence and post-harvest persistence of *Ec*O157 under organic and conventional spinach production.

Methods: Spinach was cultivated under organic and conventional conditions in San Benito and Monterey County, California. Standard management practices were followed with the subsequent modifications: nitrogen management, field location and spinach cultivar. Pre-harvest inoculum doses were 1.45 and 3.4 CFU/m^2 of planted bed. Three plant developmental stages were evaluated. Harvested product was stored for up to 14 days at 5 °C. Survival of *Ec*O157 in soil under both cultivation systems was also monitored over 45 days after incorporation of inoculated plants (at varying C/N in crop residue) sprayed with log 4 CFU/ml. Post-harvest inoculations were done with attenuated *Ec*O157 and four different pathogenic*Ec*O157 strains at log 2 CFU/ml and stored for 6 days at 15 °C.

Results: High N grown leaves supported greater survival of *Ec*O157 irrespective of farm practice ($P < 0.05$). Greater survival of *Ec*O157 was observed in older leaves irrespective of cropping system ($P < 0.05$). Prevalence of *Ec*O157, applied pre-harvest, at 5 °C was observed during 14 days of storage with greater number of positive samples for high inoculum doses ($P < 0.05$). Greater survival was observed for pathogenic than non-homologous attenuated strains (60 of 80 vs. 34 of 80, respectively; $P < 0.05$) applied post-harvest. Pathogenic strains with greater attachment traits were recovered in higher numbers than other test isolates (52 of 80 vs. 25 of 80, respectively; $P < 0.05$). Survival of *Ec*O157 in both systems was prolonged in the presence of spinach residues (31 vs. 45 days, $P < 0.05$) irrespective of C/N ratio.

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Significance: Risk assessment of farm practices under organic and conventional spinach should focus prevention strategies on optimal N fertilization and minimizing contamination potential close to harvest.

T4-05 Prevalence of Foodborne Pathogens in Fresh Produce in the U.S.-Data from the USDA Microbiological Data Program (MDP)

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Introduction: The Microbiological Data Program (MDP), initiated by USDA-Agricultural Marketing Service (AMS) in 2001, provides data on targeted pathogens in select fruit and vegetables. This data is available to federal, state agencies and the produce industry for food safety decision making purposes, risk modeling, and trend analysis. This program coordinates its activities with CDC, FDA and the National Agricultural Statistics Service (NASS).

Purpose: To provide information on the evolution of the program, present data on sampling, number and type of pathogens isolated, serotypes, PFGE data, and antimicrobial resistance characteristics.

Methods: Different produce commodities were collected every month from 11 participating states to represent about 50 percent of the population and different regions in the country. Samples were analyzed in eight (8) state laboratories for *Salmonella* spp., *E.coli* O157: H7, Non-O157 Shiga toxin *E.coli* (STEC) and *Listeria monocytogenes*.

Results: From 2001-2008, there were 0.05% (38/74,988) positives for *Salmonella* and 0% (0/42,784) for *E.coli* O157: H7. From 2009-2011, the positive rate trended upward following method improvements to 0.15% (80/52,126) for*Salmonella* and 0.01% (2/14,756) for *E.coli* O157: H7. During these two periods, the program detected 0.11% (25/21,515) vs. 0.14% (70/49,862) non-O157 STEC. Testing for *L. monocytogenes* began in October 2011 and has detected 0.4% (4/1,015) positives. Cilantro and spinach samples had the highest number of positives for *Salmonella* spp. and non-O157 STEC and a wide range of serotypes were detected. The Pulsed Field Gel Electrophoresis (PFGE) patterns of the isolates were uploaded to CDC's PulseNetfacilitating early identification and investigation of common source outbreaks. The antimicrobial resistance characteristics were reported to CDC's NARMS database. The FDA used MDP data for investigations, initiating voluntary recalls, and developing guidance letters to growers.

Significance: MDP collects statistically relevant microbiological data on both domestic and imported fresh produce and is the only federal program monitoring for non-O157 STEC in produce.

T4-06 Microbial Quality of Produce was Positively Associated with the Microbial Quality of Farm Worker Hands on Farms and Packing Sheds Near the U.S.-Mexico Border

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

Purpose: To address this gap, the goal of our study was to identify and quantify the role of potential environmental contaminants (soil, irrigation water, farm worker hands) on produce contamination.

Methods: 161 produce (cantaloupe, jalapeño, tomatoes) and matched 89 irrigation water, 55 soil, and 106 farm worker hand rinse samples were collected from 14 farms on the Mexican side of the U.S.-Mexico border. Samples were quantified for generic *E. coli*, Enterococcus, and coliforms. Chi-square tests and logistic regression models (prevalence), Spearman's correlations (non-normal data), and linear regression models (levels) were used to assess magnitude of association.

Results: In general, farm worker hands were significantly more likely to be positive for bacterial indicators, and at higher levels than soil and water. The presence of *E. coli*, but not Enterococcus or coliforms, was significantly associated between hands and produce (OR 7.9, 95% CI [3.3-19.1]). The levels of *E. coli*(rho=0.4), Enterococcus (rho=0.5), and coliforms (rho=0.6) were significantly and highly correlated between hands and produce. These trends were also observed when analyses were stratified at the individual produce type level. Unlike hands, the presence or levels of indicators on soil and water samples were not significantly and positively associated with the presence or levels of indicators on produce samples.

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

T4-07 Survival and Detection of *Escherichia coli* O157:H7 and *Salmonella enterica* Surrogates on Field Grown Mini-greens

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Introduction: Leafy greens, including diverse baby-leaf types, have been implicated in numerous outbreaks and recalls associated with human pathogens. Understanding their population dynamics during crop production and strategies for accurate detection is needed to improve and establish optimal food safety standards and audit criteria.

Purpose: To monitor the population dynamics of low levels of *E. coli* O157:H7 and *S. enterica*on the phyllosphere of diverse mini-greens during field production and identify factors that influence detection after environmental exposure.

Methods: Five varieties of lettuce were inoculated with two inoculum doses (log 4 and log 6 CFU/ml) of attenuated strains of *E. coli* O157:H7 (PTVS155) and *S. enterica* sv. Typhimurium (PTVS177) during field production. Changes in population were monitored after 12 h, 3 and 10 days post inoculation using selective quantification and/or enrichment. Additionally, molecular detection with commercial kits using different sample sizes (25 to 375 g), enrichment media composition and ratio, and time of enrichment in field inoculated samples, as well as *in vitro*inoculated lettuce samples, were compared.

Results: Populations of *E. coli* O157:H7 and *S. enterica* significantly declined 12 h post inoculation; surviving populations were recovered after selective enrichment. Prevalence of both pathogens was reduced with the time of exposure in the open environment. Overall, better fitness on the phyllosphere was observed for *S. enterica* than the applied *E. coli* O157:H7. Detection assays demonstrated that large sample mass (375 g) in combination with low ratio (plant material:enrichment broth), short enrichment periods (<12 h), and non-green pigmentation of leaves increased the occurrence of false negatives. A lower percentage of positive samples was detected in field-inoculated than *in vitro*inoculated samples of the same leaf tissues.

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Significance: Validation of molecular detection platforms for pathogens on fresh produce should be done in a context that considers abiotic and biotic phyllosphere interactions on the target pathogen as well as sample mass and processing matrix.

T4-08 *Escherichia coli* Persistence on Broccoli, Cauliflower and Chinese Cabbage Crops after Irrigation

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Introduction: Irrigation water is recognized as a potential source of human pathogens. However, few studies were conducted on the persistence of indicator microorganisms on crops after irrigation under field conditions.

Purpose: The aim of this project was to evaluate the persistence of *Escherichia coli*, used as a fecal contamination indicator, on *Brassicaceae*crops after irrigation with contaminated water.

Methods: Experimental plots were set in a completely randomized design including 3 crops (broccoli, cauliflower, and Chinese cabbage) each repeated four times. Plots were spray-irrigated with water from an adjacent stream having an *E. coli* content of 1,151 CFU/100 ml at irrigation time. Three vegetables were sampled in each plot before irrigation and at different times after irrigation (1 hour, and 1, 2, 3, 4, 5, 6, 8, 9, 10, 19 days) for *E. coli* detection by enrichment and the determination of *E. coli* populations. A logistic regression of *E. coli*prevalence on time was fit to data using the SAS GLIMMIX procedure for the estimation of the equation parameters.

Results: *E. coli* could be quantified in only 5 % of the vegetables samples taken after irrigation, with bacterial contents varying between 10 and 130 CFU/g (n=396). *E. coli* was detected by enrichment in 28 % of the samples. Statistical analysis showed a significant impact of the crop and the delay between irrigation and harvest (and their interaction) on *E. coli* prevalence. The logit of *E. coli*prevalence decrease rates were estimated at -0.0336, -0.0114, and -0.0044 in broccoli, cauliflower and Chinese cabbage, respectively.

Significance: Results emphasize the importance of the crop and the delay between irrigation and harvest on *E. coli* prevalence on *Brassicaceae* crops.

T4-09 Effect of Spinach Cultivar and Strain Variation on Survival of *Escherichia coli* O157:H7 on Spinach Leaves

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Introduction: *Escherichia coli* O157:H7 outbreaks of infections associated with the consumption of fresh produce have increased in recent years. Bacterial cell surface appendages such as curli and the spinach leaf structure topography influence pathogen attachment and subsequent survival on spinach plants.

Purpose: Role of curli and cellulose in *E. coli* O157:H7 attachment and persistence to different spinach cultivars was evaluated.

Methods: Four organic spinach cultivars—Emilia, Lazio, Space, and Waitiki—were grown in sandy loam soil (3% organic matter). Leaves of four weeks old spinach plants were spot inoculated (100 µl in 5µl droplets, 5 log CFU/leaf) with *E. coli* O157:H7 strain EDL 933 and its isogenic mutants deficient in curli (EDL933D*csgA*), cellulose (EDL933D*bcsA*), or both (EDL933D*csgA*/D*bcsA*). Four replicates of each plant shoot were analyzed for 14 days for *E. coli*O157:H7 by spiral plating and MPN enrichment procedure. Leaf structure of spinach cultivars were observed under Low Temperature Scanning Electron (LT-SEM) and Variable Pressure Electron microscopy (VP-SEM).

Results: Curli expressing *E. coli* O157:H7 strains persisted at significantly higher level for 14 days on each spinach cultivar whereas curli deficient strains were undetectable after 7 days. Lack of cellulose expression did not affect its ability to persist on spinach leaves. The effect of spinach cultivar on *E. coli* O157:H7 persistence was not evident on days 0 and 1; however, significantly higher populations of *E. coli* O157:H7 were recovered from Waitiki cultivar during subsequent sampling on 7 and 14 days. Observation of the leave surface under LT-SEM did not found major differences in leaf structure among cultivars. However, quantitative topography analysis of the leave surface under VP-SEM revealed significantly greater surface roughness in Waitiki cultivar leaves.

Significance: Selection of spinach cultivars that restrict pathogen attachment to its surface can be useful intervention strategy in good agricultural practices to reduce potential produce-borne outbreaks.

T4-10 Effect of Storage Temperature on the Survival and Growth of *Listeria monocytogenes* Populations in the Presence of Indigenous Surface Microflora of Fresh-cut Cantaloupes

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Introduction: The most recent outbreak of listerosis linked to consumption of fresh-cut cantaloupes contaminated by *L. monocytogenes* suggests the need to investigate the behavior of *Listeria monocytogenes*in the presence of native microflora of cantaloupe pieces during storage.

Purpose: The behavior of *L. monocytogenes*in the presence of native microflora of cantaloupe pieces during storage and the effect of waiting period before refrigeration on microbial populations on fresh-cut cantaloupes was investigated.

Methods: Whole cantaloupes were inoculated with *L. monocytogenes* (10^8 CFU/ml of suspension) for 10 min and air dried in a biosafety cabinet for 1 h and then were treated (unwashed, water washed and 2.5 % hydrogen peroxide (H_2O_2)). Fresh-cut pieces (~3 cm) prepared from these melons were left at 5 °C and 10 °C for 72 h and room temperature (20 °C) for 48 h. Some fresh-cut pieces were stored at 5 °C after 2 h and 4 h of storage at 20 °C. Microbial populations of fresh-cut pieces were determined immediately by the plate count or enrichment method after preparation and during storage.

Results: Aerobic mesophilic bacteria and yeast and mold of whole melon and inoculated populations of *L. monocytogenes* on cantaloupe rind surfaces averaged 6.5 log CFU/cm^2, 3.3 log CFU/cm^2 and 4.6 log CFU/cm^2, respectively. Among the treatments, only H_2O_2 (2.5%) reduced the aerobic mesophilic bacteria, yeast and mold and *L. monocytogenes* populations to 2.8, 1.3 and 1.8 log CFU/cm^2, respectively. The populations of *L. monocytogenes* transferred from melon rinds to fresh-cut pieces were below detection (< 2 CFU/g). Storage temperatures enhanced the lag phases and growth of *L. monocytogenes* as evidenced by the increase in generation time, implying that these conditions could be a risk factor for produce contamination. Fresh-cut pieces with low populations of *L. monocytogenes* and approximately 2.8 log CFU/g mesophilic aerobic bacteria had an extended lag phase of 6 h at 10 °C and 4 h at 20 °C before growth of the pathogen could be detected.

Significance: These results suggest that a waiting period of 4 h at 20 °C before refrigeration of prepared fresh-cut cantaloupes enhanced growth of indigenous microflora and *L. monocytogenes* transferred to fresh-cut pieces suggesting the need for immediate refrigeration of prepared fresh-cut pieces at 5 °C.

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T4-11 Evaluation of Infrared Technology for Temperature Audits of Bagged Leafy-green Produce in Retail Cold-chain Display

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Introduction: Packaged, fresh-cut leafy greens have been associated with several foodborne illness outbreaks. The revised FDA Food Code now includes cut leafy greens among foods that require time and temperature control for safety at or below 41°F (5 °C). Commercial retail establishments commonly display leafy greens in open refrigerated display cases and use hand-held infrared "non-contact" thermometers to ensure compliance with FDA temperature regulations.

Purpose: The purpose of this study is to determine the accuracy of infrared technology in performing temperature audits of packaged cut leafy greens in retail cold-chain display.

Methods: Thirty 'point-of-sale' commercially-bagged baby spinach products were purchased at retail. Each bag was manually opened and individual temperature dataloggers were placed in the middle of the product. The bags were resealed to original specifications and placed in the outermost shelf locations in a new, commercial, 12-foot refrigerated open-display case operating at the industry-recommended thermostatically-controlled temperature-defrost schedule. Temperature measurements from each bag were also collected manually using a hand-held infrared thermometer at random times through the daily operation (including defrost periods) of the retail display case. Thermograph profiles obtained from each bag, shelf, and the ambient conditions were also determined.

Results: Bland-Altman and regression analysis of the non-contact infrared and the internal bag temperature datalogger data showed a significant bias between the measurements; the infrared technology overestimated the leafy green product temperature by approximately 3°F. Furthermore, when temperatures were obtained at the end of the display case defrost cycle (maximum case thermostat temperatures), the mean difference between the two measurement methods dramatically increased and the internal produce temperatures were overestimated by greater than 9°F using infrared technology.

Significance: Temperature monitoring and auditing must reflect the actual product temperatures. Infrared technology is simple, easy and widely used, but does not accurately reflect internal product temperature of bagged baby spinach.

T4-12 Monte Carlo Simulation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* Growth in Bagged Salad Greens during Commercial Transport, Retail Storage and Display

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Introduction: The probability distribution for growth of bacterial pathogens in leafy greens during commercial transport and retail sale is one of the major data gaps in current risk assessments.

Purpose: Using a series of commercial time/temperature profiles collected across the United States, the goal of this study was to simulate the probability distributions for growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes*in commercially bagged salad greens during transport, retail storage and display.

Methods: Over a 16-month period, a series of time/temperature profiles were obtained from bagged salad greens in 16 transport routes covering four geographic regions (432 profiles), as well as during retail storage (4,867 profiles) and display (3,799 profiles) at nine supermarkets. Five different time/temperature profiles collected during 2 – 3 days of transport, 1 and 3 days of cold room storage and 3 days of retail display (including the best and worst scenarios) were then duplicated in a programmable incubator to assess *E. coli* O157:H7 and *L. monocytogenes*growth in commercial bags of a Romaine lettuce salad mix. Microbial growth predictions using the Baranyi/Ratkowsky model were validated by comparing the root mean square error (RMSE) and bias between the laboratory growth data and model predictions. Monte Carlo simulations (~100,000 iterations) were then performed to calculate the probability distribution of microbial growth from a total of 432 × 4867 × 3799 (7,987,564,656) scenarios during transport, cold room storage, and retail display.

Results: Using inoculated bags of retail salad, *E. coli* O157:H7 populations increased a maximum of 0, 0.6, and 0.3 log CFU/g during transport, retail storage, and display, respectively, with *L. monocytogenes* populations increasing 0.6, 1.9, 1.1 log CFU/g during the same three periods. The RMSEs between the experimental and predicted populations were all acceptable (*E. coli* O157:H7 < 0.8 log CFU/g, *Listeria* < 1.2 log CFU/g). Based on the simulation results, both pathogens generally increased < 1 log CFU/g. Storing the product for 1 and 3 days before display yielded overall growth (> 1 log CFU/g) probabilities of 0.02 and 0.34 for *Listeria*, respectively.

Significance: This large-scale US study—the first using commercial time/temperature profiles to assess the microbial risk of leafy greens during transport, retail storage, and display—should be useful in filling some of the data gaps in current risk assessments for leafy greens.

T5-01 Thermal Tolerance of Shiga Toxin-producing *Escherichia coli* (STEC) Strains in Ground Beef of Varying Fat Levels

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Introduction: In 2011, the United States Department of Agriculture declared six serogroups of non-O157 STEC as adulterants: O26, O45, O103, O111, O121 and O145 in non-intact raw ground beef. An understanding of the thermotolerance of non-O157 STEC in ground beef of varying fat levels is important in establishing the efficacy of industry interventions against these new adulterants.

Purpose: We compared the thermotolerance of two representative strains from each of the six serogroups with reference O157:H7 strains at 65.5°C in 15% and 27% fat ground beef. Reference O157:H7 strains included four heat tolerant beef-trim isolates and an outbreak-linked strain (ATCC 43895). Thermal tolerance of a mutant strain of O157:H7 lacking the gene for sigma factor *rpoS* and a lactic acid bacterium (LAB) was also determined. The LAB (*Pediococcus acidilactici*) has shown to be an effective surrogate for O157:H7 in beef thermal processing.

Methods: Small Whirl-Pak bags containing 25 g preheated ground beef (65.5°C) were inoculated with 1 ml aliquots of stationary-phase culture (~10^9 CFU/ml), and immediately transferred to a shaking water-bath (65.5°C). At each sampling time (0 - 180 s), one bag of meat was removed and held on ice to reach ≤4°C. The meat was everted into a stomacher bag and stomached with 99 ml Butterfield's Phosphate Diluent (BPD) for 30s. Serial dilutions were made in BPD and viable cells enumerated by plating on modified Eosin Methylene Blue agar (37°C for 24h). $D_{65.5°C}$-values were calculated from log CFU/g data, and compared using ANOVA (n=3 at each fat level).

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Results: At a 15% fat level, $D_{65.5°C}$-values ranged from 69.54 s (*E. coli* O145:H-) to 37.75 s (*E. coli* O121:H19), though there were no significant differences between the strains ($P \geq 0.05$). For 27% fat, D-values ranged from 78.74 s (*E. coli*O157:H7 beef trim isolate) to 35.21 s (ATCC 43895).

Significance: These results suggest that levels of fat in meat affect the survival of the pathogen and thermotolerance of the pathogen is affected by the strain in question. This data is important in establishing industry intervention protocols.

T5-02 Validation Study for the Detection of *Escherichia coli* O157:H7 from Ground Beef and Beef Trimmings

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Introduction: Ruminants in general and cattle specifically are the main reservoir for Shiga toxin-producing *Escherichia coli* (STEC) and pose a threat to consumers via food. *Escherichia (E.) coli* O157:H7 were described as protopathotype of STEC, other STEC serotypes like O111, O26, O103, O145, O45 and O121 are emerging. Due to the severe illnesses caused by these pathogens, a fast and reliable detection method to ensure food safety is required.

Purpose: The study presented shows an accelerated, validated method for screening and isolation of STEC as alternative to the USDA FSIS reference method MLG 5.04.

Methods: Beef trim (n=60) and ground beef (n=60) samples with 375 g weight were artificially spiked with approximately 0.7 CFU/sample cold-stressed *E. coli* O157:H7. The enrichment extended for 8 hours (beef trims), respectively, 10 hours (ground beef) in RapidCult™ *E. coli* Enrichment broth and was used for screening with Foodproof® *E. coli* O157 Detection Kit in Real Time (RT)-PCR and also Lateral Flow based via Singlepath® *E. coli*O157. Following Immunomagnetic Separation, the identification of suspect colonies on CT-SMAC agar was performed by RT-PCR, Singlepath® test system and examination of Shiga toxin production with Duopath® Verotoxins.

Results: Without any false negative results, the method showed a sensitivity of 100 %; the specificity varied by 70-100 % depending on method cascade and matrix. The new enrichment method enables a screening of 375 g (pooled) samples with results within 14 hours and a completed confirmation already after 3 days. Additional data show the detectability of other monitored STEC such as O111, O26, O103, O145, O45 and O121 from 25 g samples of ground beef after only 8 h incubation time.

Significance: The results demonstrate an alternative method to the MLG protocols allowing a faster *E. coli* O157:H7 and STEC screening for improvement of food safety. In addition, this method can also be used for the detection of other STEC from ground beef.

T5-03 Quality System Implementation in Mexican Exporting Pork Packers

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Introduction: HACCP system implementation has become a baseline in the international trade of food commodities.

Purpose: The study addresses the level of adoption of ISO 9000 and the HACCP system within certified (TIF) Mexican pork meat packers with export sales along with the identification of benefits and restraints associated to these quality systems.

Methods: The 21 registered TIF packers were approached and 95.2% of them filled out the structured questionnaire. Information obtained was on implementing and operating costs, benefits, plant and specific processes characteristics. Data was subjected to statistical analyses by univariate and tabulate procedures of SAS.

Results: All TIF packers had the HACCP System operating in their plants, while only 30% of them had also ISO 9000 in full operation. On average, 75% of the production is exported to a market of 13 countries and 15% goes to a high-income sector of the Mexican society. Access to international market, better product quality, and lower customer complaints were the three major reasons for implementing the HACCP system within the plants. Staff training was pointed out as the major constraint and microbiological testing as the highest cost of operation. The main benefit was a competitive position as supplier in the international market due to lower microbial counts in the products sold.

Significance: TIF Mexican pork packers are aware of the commercial benefits derived from the implementation of food safety protocols in their plants and operating processes.

T5-04 Survival of *Salmonella* on Cooked Pig Ear Pet Treats

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Introduction: Pet treats including pig ears have been implicated as vehicles of human salmonellosis. Although pig ears are cooked to temperatures that are lethal to *Salmonella*, there is a possibility of post-cooking recontamination prior to or during packaging. Therefore, behavior of the pathogen on these very low moisture pet treats is of interest.

Purpose: The objective of this study was to measure the survival of two *Salmonella*serovars on two pig ear products at refrigeration and room temperature.

Methods: Natural (a_w = 0.256) and smoked (a_w = 0.306) pig ears were spot inoculated separately with *Salmonella*Newport and Typhimurium dt104 at ca. 6 log CFU/sample, sealed in plastic bags, and stored at 4.4 and 22 °C. Surviving populations of the inocula were enumerated by rinsing pig ears with 50 ml of peptone-tween-salt diluent and plating on tryptic soy agar overlaid with a thin layer of Hektoen enteric agar. Average log population declines that occurred between 0 and 14 days of storage were analyzed by two-way, repeated measures ANOVA.

Results: Storage temperature significantly ($P < 0.05$) influenced decline of *Salmonella*, but serovar, type of pig ear, and two-factor interactions did not. At 22 °C decline in *Salmonella* Newport and Typhimurium dt104 on natural pig ears was 2.65 and 0.81 log, respectively, while both serovars declined by >1 log on smoked pig ears. At 4.4 °C decline of the *Salmonella*serovars was lower, ranging from 0.27 to 0.90 on the two pig ear types.

Significance: Viable *Salmonella* slowly declines on very low a_w refrigerated pet treats and more rapidly declines at room temperature. This information may be useful for pet treat safety assessments and for determining target inactivation levels for post-lethality treatments.

T5-05 Effect of Non-pharmaceutical Compounds on *Salmonella* Shedding and Colonization in Broiler Chickens

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Introduction: Feed withdrawal (8 to 12 h preslaughter) reduces the amount of feces in the bird gut and therefore, reduces the potential risk of carcasses being contaminated with *Salmonella* during processing. However, feed withdrawal has been shown to increase the populations of *Salmonella*in ceca and crops of broilers.

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Purpose: The objectives of this study were to determine the effect of non-pharmaceutical compounds on performance, mortality and prevalence of *Salmonella* Heidelberg (*SH*) in broilers challenged with *SH*.

Methods: Chicks were randomly assigned to water treatments containing organic acids, essential oils, lactic acid, levulinic acid plus sodium dodecyl sulfate, or no added compounds (control). Treatments were administered in drinking water on 0 to 7 and 35 to 42 days. One-half of the chicks were challenged with *SH* and placed in pens with unchallenged chicks on day one. Performance and mortality were determined during the 42-day study. Prevalence of *SH*was determined on drag swabs (0, 14 and 42 days) and in the ceca and crops (42 days).

Results: Broilers receiving essential oils had significantly greater gain and lower mortality than other treatments. *Salmonella* Heidelberg was absent from drag swabs on day 0, but present at 14 and 42 days. Challenged broilers receiving essential oils and unchallenged broilers receiving essential oils and lactic acid had significantly lower *SH*in crops than other treatments.

Significance: Essential oils may control *SH* contamination in crops of broilers when administered in drinking water. Furthermore, essential oils may be an alternative to organic acids in broilers as preslaughter intervention as *SH* reduction treatment.

T5-06 A Microbiological Comparison of Poultry Products Obtained from Farmers' Markets and Supermarkets in Pennsylvania

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Developing Scientist Competitor

Introduction: The popularity of farmers' markets continues to rise in the United States. Raw poultry products sold at farmers' markets are of particular concern due to the USDA inspection exemption status afforded to many poultry vendors. Currently, little to no data have demonstrated the microbiological profile of poultry sold at farmers' markets and/or compared to conventionally-processed, organic and non-organic poultry sold in supermarkets.

Purpose: The purpose of this study was to determine the presence/absence of foodborne pathogens as well as hygiene indicators in broilers purchased at farmers' market and conventionally-processed, organic and non-organic broilers sold in supermarkets. Additionally, a needs assessment was developed and disseminated to evaluate the food safety knowledge and attitudes of poultry vendors at farmers' markets.

Methods: Whole broilers from farmers' markets and supermarkets throughout Pennsylvania were obtained and transported at 4°C until further analysis. Each broiler was rinsed and levels of aerobic plate counts (APC), generic *Escherichia coli* and total coliforms were measured. Resulting samples also were evaluated for presence/absence of *Campylobacter* spp. and *Salmonella*spp. following standard culture and confirmation methods. A food safety needs assessment was additionally administered to individual vendors.

Results: Results demonstrated that 28% (28/100) and 90% (90/100) of broilers from farmers' markets, 20% (10/50) and 28% (14/50) of conventionally-processed organic, and 8% (4/50) and 52% (26/50) of non-organic broilers, were positive for *Salmonella* spp. and *Campylobacter*spp., respectively. The needs assessment survey revealed that vendors exhibited critical gaps in the regulatory requirements as well as food safety knowledge and practices during poultry processing.

Significance: These data suggest that poultry purchased from farmers' markets in Pennsylvania were more likely to be contaminated with *Salmonella* spp. and *Campylobacter* spp., as compared to conventionally-processed poultry sold at supermarkets. This study also demonstrates the need to develop food safety training for poultry vendors at farmers' markets in order to improve the safety of these products for public consumption.

T5-07 Reductions in Pathogens and Quality Characteristics of Poultry Carcasses Treated with Various Antimicrobials in a Finishing Chiller

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Developing Scientist Competitor

Introduction: With the implementation of more stringent regulatory guidelines, it is necessary for processors to employ new or additional pathogen intervention strategies for more effective control of *Salmonella* and *Campylobacter*throughout poultry processing. New innovations in poultry include post-chill decontamination tanks. Additionally, because treatment with antimicrobials can affect the organoleptic properties of a product, it is important to determine the quality aspects of carcasses treated with antimicrobials.

Purpose: The objectives of this study were to determine the efficacy of various antimicrobials added to the finishing chiller in reduction of *Salmonella* and *Campylobacter*and to evaluate any associated effects of the various antimicrobials on quality characteristics of chicken breast meat.

Methods: Five chill water treatments consisting of 0.04% chlorine, 0.04%, 0.1% PAA, 0.1%, or 0.5% lysozyme were examined using a finishing chiller. The skin of carcasses was inoculated with *Salmonella* Typhimurium (10^6 CFU/mL) and*Campylobacter jejuni* (10^6 CFU/mL). Following a 20 min attachment time, carcasses were dipped into the finishing chiller for 20 s. Individual birds were then placed into a sterile rinse bag and rinsed with 200 ml buffered peptone water for 1 min. Serial dilutions were performed and 0.1 ml was spread plated on differential media for enumeration of *Salmonella* and *Campylobacter*. Non-inoculated chicken breast meat from each treatment was used for sensory analysis.

Results: Treatment with 0.04% and 0.1% PAA was found to be most effective in decreasing *Salmonella* and *Campylobacter*. Chlorine treatment at 0.004% and lysozyme applied at 0.1% and 0.5% were found to be less effective and resulted in less than a one-log reduction when compared to controls. Treatment with the various antimicrobials was not found to have negative impacts on sensory attributes.

Significance: Utilizing PAA in a finishing chiller is an effective application for reducing *Salmonella* and *Campylobacter* on carcasses while maintaining product quality.

T5-08 Effect of Vaccines in Commercial Layer Chickens against Various *Salmonella* Serovars

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Introduction: Salmonellae are of major public health concern and transmission to humans is often attributed to poultry meat and eggs. Vaccination represents one strategy for managing *Salmonella* in poultry production. A previous study demonstrated efficacy of an inactivated trivalent vaccine in reducing the carriage in meat chickens of prevalent *Salmonella* serovars.

Purpose: To evaluate the efficacy of attenuated and inactivated vaccines, alone or in combination, administered by various routes, for reduction of colonization of layer hens by *Salmonella*.

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Methods: Live, attenuated (Bioproperties Vaxsafe ST) and inactivated (Intervet) vaccines against *Salmonella* were administered, alone or in combination, using four different vaccination regimes, to Hyline brown-egg-laying chickens. Vaccines were applied up to 18 weeks of age. The birds were challenged with *Salmonella* Typhimurium, *S.* Infantis or *S.* Virchow at ~ 108 colony forming units (CFU) per bird of at 10, 16 and 22 weeks of age. Three weeks after challenge, the birds were euthanized and their ceca cultured for salmonellae. A protective index was determined by comparing populations in vaccinated and control birds. Blood was collected and titres against *S.* Typhimurium were determined using an ELISA.

Results: Protection was demonstrated against *S.* Typhimurium and *S.* Infantis by both vaccines with some protection afforded against *S.* Virchow by some vaccine combinations. The best overall protection was delivered by the combination of subcutaneous injection of the live vaccine at six weeks followed by an intramuscular injection of the inactivated vaccine at 12 weeks of age. Vaccination induced an antibody response that was stronger following two vaccinations. It was concluded that there was an association between high antibody level and protection against *Salmonella* colonization.

Significance: The results suggest that use of inactivated and attenuated vaccines, particularly in combination, will be useful in an integrated management strategy for control of *Salmonella* in poultry production.

T5-09 The Effects of Salinity on the *In Vitro* Growth and Survival of Pathogenic *Vibrio* Species

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Introduction: *Vibrio vulnificus* (Vv), *V. parahaemolyticus* (Vp) and *V. cholerae* (Vc) cause >75% of seafood-related bacterial infections in the U.S. The transport or relay of oysters to areas where conditions are unfavorable to *Vibrio*survival is under consideration and generally coincides with increased salinity.

Purpose: The effects of salinity on the *in vitro* growth and survival of pathogenic *Vibrios* were investigated.

Methods: Individual and co-inoculated cultures were assessed by plate count and multiplex QPCR (DuPont BAX) for growth in nutrient media at various salinities (5, 10, 20, 30, 35 ppt) and for survival in artificial seawater (ASW) following salinity upshifts (20 to 30 ppt or 20 to 35 ppt) at different temperatures (23 and 30 °C).

Results: The salinity range for optimum growth yield at 6 h differed for Vp (10 to 35 ppt); Vc (5 to10 ppt); and Vv (10 ppt). Higher yield of Vp in competitive culture at 35 ppt ($P = 0.03$) was the only observed difference for competitive vs. individual growth. Salinity upshifts did not alter survival of Vp after 32 days in ASW; whereas, Vv and Vc showed ca. 2.0 log CFU/ml reduction ($P < 0.04$) by plate count following upshifts from 20 to 30 ppt (but not 20 to 35 ppt) at 23°C (but not 30°C). However, reductions were not confirmed by QPCR, suggesting upshifts may induce a viable but nonculturable state. The only difference in competitive vs. individual survival in ASW was a greater reduction ($P = 0.03$) of Vv in the competitive sample following an upshift from 20 to 30 ppt at 23°C.

Significance: These quantitative data on the effects of salinity and temperature on growth and survival of *Vibrio* species suggest that design parameters for oyster relay practices should be carefully evaluated in order to optimize conditions for the reduction of *Vibrio* contamination of oysters.

T5-10 A Predictive Model for the Decontamination Effect of Lactic Acid and Chitosan on *Vibrio parahaeomolyticus* in Shrimp

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Developing Scientist Competitor

Introduction: *Vibrio parahaeomolyticus* is a major causative agent of human gastroenteritis in seafood including shrimp. Lactic acid and chitosan are natural antimicrobials for food decontamination without risk to human health. However, limited information is available on the combined use of these two natural decontaminants to eliminate or reduce *V. parahaeomolyticus*in shrimp.

Purpose: The purpose of this study was to evaluate the combined effect of lactic acid and chitosan on reducing *V. parahaeomolyticus* in shrimp during washing process.

Methods: A 3-factor response surface model based on the Box-Behnken design was developed to evaluated the effect of lactic acid concentration (1.5, 2, and 2.5% v/v), chitosan concentration (0.8, 1.2, and 1.6% w/v) and washing time (10, 15, and 20 min) on reduction of *V. parahaeomolyticus* in inoculated raw shrimp. The model was validated with 10 random additional treatments within the model range.

Results: The result indicated that the model was significant ($R^2 = 0.98$, $P < 0.001$) and achieved 1.35 - 2.42 log CFU/g bacterial reduction in raw shrimp after different treatment combinations. Lactic acid, chitosan, and washing time all exhibited an individual significant effect on bacterial reduction ($P < 0.05$), and the interaction between lactic acid and chitosan was significant ($P < 0.05$), indicating improved antimicrobial effect with their combination. Bias factor (B_f) and accuracy factor (A_f) calculated from the validation tests were 1.06 and 1.03, respectively, suggesting a good performance of the model.

Significance: The combination of lactic acid with chitosan could be an alternative decontaminant for shrimp postharvest washing, and the predictive model could be used to design appropriate combinations of lactic acid and chitosan to achieve desirable *V. parahaeomolyticus* reduction.

T5-11 Effect of *Lactobacillus acidophilus* La-5 Fraction on the Presence of *Salmonella* Typhimurium in Pigs

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Introduction: The persistence of *Salmonella* in pigs results in asymptomatic carriers constituting a major source of salmonellosis in humans. The capacity of probiotics to inhibit the virulence expression of pathogenic bacteria offers potential novel therapeutic approaches to combat this pathogen. Previous studies in our laboratory have shown that a *L. acidophilus* La-5 fraction interferes with the pathogenicity of *Salmonella*Typhimurium in vitro.

Purpose: The aim of the present work was to investigate the effect of a probiotic *L. acidophilus* La-5 fraction on pigs to protect against *Salmonella* infection.

Methods: Six-week-old piglets were acclimatized at the animal facility for 14 days prior to inoculation with *Salmonella* Typhimurium SA941256. Two different protein concentrations of the *L. acidophilus* La-5 fraction were administered to two groups of 6 *Salmonella*-free piglets. After 7 days on this feeding regime, piglets were inoculated with 1.5×10^7 CFU of *S.* Typhimurium and the feeding regime was continued for 4 days post-infection. Bacteriological and molecular analyses were performed to detect *S.* Typhimurium in rectal swabs 4 days post-infection and in different tissues after euthanization.

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Results: *Salmonella* was not present in rectal swabs after 48 h post-infection in piglets fed with the probiotic fraction containing a high protein concentration. Moreover, the bacterium was not found in the ileum and jejunum of these piglets. No statistically significant differences ($P < 0.05$) were found in the numbers of *Salmonella* in caecum and the contents of jejunum, ileum and caecum compared to non-treated piglets. However, the *Salmonella* amount in the contents was 1-log CFU less compared to non-treated piglets. *Salmonella* was detected by real-time PCR in the ileum of non-treated animals but not in piglets fed with the high-protein probiotic fraction.

Significance: Administration of bioactive peptides produced by probiotics reduces *Salmonella* carriage by pigs and may help reduce the burden of foodborne illness resulting from contamination of pork.

T6-01 Thermal Inactivation of Stationary Phase and Acid Adapted Shiga Toxin-producing *Escherichia coli* in Single-strength Orange Juice

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Developing Scientist Competitor

Introduction: Thermal inactivation parameters of stationary phase and acid adapted pathogens, primarily as cocktails of multiple strains,have been studied in various juice products.

Purpose: The objective of this study was to evaluate the heat resistance of stationary phase and acid adapted Shiga toxin-producing *Escherichia coli* (STEC) in orange juice by evaluating the thermal inactivation responses of individual strains.

Methods: STEC strains of O157:H7 and O111 were evaluated. Strains were grown in TSB, supplemented with 1% glucose for acid adaption, and inoculated into single-strength pasteurized orange juice without pulp. Inoculated juice was sealed into microcapillary tubes. Tubes were immersed into water baths at 56, 58, and 60°C, removed at predetermined time intervals, and placed immediately onto ice. Thermally treated and sterilized tubes were crushed in 0.1% peptone using sterile glass rod for microbiological analysis. STEC populations were enumerated on a tryptic soy agar supplemented with 0.1% sodium pyruvate.

Results: D-values for acid adapted and non-adapted *E. coli* O157:H7 at 56, 58, and 60°C are 2.78 ± 0.01 and 2.72 ± 0.01 min, 1.38 ± 0.02 and 1.27 ± 0.03 min, and 0.60 ± 0.05 and 0.52 ± 0.05 min, respectively. A similar trend was seen for *E. coli* O111, where D-values for acid adapted and non-adapted cells at 56, 58, and 60°C are 3.13 ± 0.06 and 2.90 ± 0.01 min, 1.49 ± 0.03 and 1.37 ± 0.04 min, and 0.62 ± 0.04 and 0.54 ± 0.05 min, respectively. D-values for *E. coli*O111 are higher at these temperatures than those of *E. coli* O157:H7. Acid adapted STEC have higher D-values than non adapted cells at all temperatures. D-value differences between acid adapted and non-adapted *E. coli* O111 cells were greater than in *E. coli* O157:H7 at 56 and 58°C.

Significance: Currently, all available D-values for STEC in orange juice were obtained using strain cocktails. Evaluation of individual strains using the microcapillary tubes will allow us to understand strain to strain variability.

T6-02 Human Norovirus Surrogate Reduction in Milk and Juice Blends by High Pressure Homogenization

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Developing Scientist Competitor

Introduction: Novel processing technologies such as high pressure homogenization (HPH) for the inactivation of foodborne viruses in fluids that also retain nutritional attributes remain in high demand.

Purpose: The objectives of this research were (1) to determine the effects of HPH alone or with an emulsifier (lecithin) on human norovirus surrogates, murine norovirus (MNV-1) and feline calicivirus (FCV-F9), in skim milk and orange juice (OJ); and (2) to determine HPH effects on FCV-F9 and MNV-1 in juice blends such as OJ blended with pomegranate juice (PJ).

Methods: Experiments were conducted with FCV-F9 and MNV-1 in duplicate with each fluid food or lecithin combination at 0, 100, 200, 250, and 300 MPa for <2 s in a high pressure homogenizer system and plaque assayed in duplicate.

Results: For FCV-F9 in milk, ≥ 4 and ~1.3 log PFU/ml reduction at 300 and 250 MPa; while ≥4 and ~1 log PFU/ml reduction in OJ were obtained only at 300 and 250 MPa, respectively. FCV-F9 was reduced to non-detectable levels at 300 MPa, and by 1.77 and 0.78 log PFU/ml at 250 MPa in OJ or milk combined with lecithin, respectively. MNV-1 in milk was reduced by ~1.3 log PFU/ml only at 300 MPa, while was reduced by ~0.8 and ~0.4 log PFU/ml in OJ at 300 and 250 MPa, respectively. MNV-1 in milk or OJ containing lecithin showed improved 1.32 or 2.5 log PFU/ml reduction, respectively at 300 MPa. In the PJ-OJ combination, FCV-F9 was completely reduced, and MNV-1 was reduced by 1.04 and 1.78 log PFU/ml at 250 MPa and 300 MPa, respectively.

Significance: These results show that HPH has potential for commercial use to inactivate foodborne viruses in juices. Lecithin and juice combinations may provide additional benefit for the inactivation of these viral surrogates.

T6-03 The Long-term Survival of *Escherichia coli* O157:H7 and *Salmonella* on Lettuce Seeds and Their Subsequent Survival and Growth on Germinating Sprouts

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Introduction: In recent years, numerous outbreaks with enteric pathogens have been associated with contaminated sprouts. Moreover, evidence suggests that the outbreaks in Germany and France in 2011 with *Escherichia coli* O104:H4 could be attributed to contaminated seeds, namely fenugreek seeds. These seeds had possibly been contaminated for up to two years. But until now, bacteriological evidence for this hypothesis is lacking. This raises the question whether enteric pathogens can survive for such a long period on seeds and resuscitate and even proliferate on the germinating sprouts.

Purpose: The present study aims to investigate the long-term survival of enteric pathogens on lettuce seeds and their subsequent survival and growth on germinating sprouts.

Methods: Lettuce seeds were inoculated at a high level with two *Salmonella* and two *E. coli* O157:H7 strains (± 8 log cfu/g). The survival of the pathogens was followed for at least one year (up to two years) using standard plating techniques on selective medium. After these two years, three methods were tested to recover the pathogens from the seeds. Last, the seeds were germinated and examined for the presence of the pathogens.

Results: Both *Salmonella* strains survived significantly better than the *E. coli* O157:H7 strains. Two years post inoculation, *Salmonella* was still present on every seed while *E. coli* O157:H7 could be recovered from only 4% to 14% of the seeds, depending on the recovery method used.

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Both pathogens were able to proliferate on the positive germinating sprouts. Densities of up to 5.92 log CFU per positive sprout were observed for *Salmonella;* for *E. coli* O157:H7 this rate was 4.41 log CFU per positive sprout.

Significance: Our study not only confirms the long-time survival of enteric pathogens on seeds but also shows that, even after long storage of the contaminated seeds, the pathogens are able to resuscitate and proliferate on the germinating sprouts. Acknowledgments: This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RF 6202).

T6-04 Development of a Phage-based Typing System to Use in Combination with Multi-locus Variable Number of Tandem Repeat Analysis (MLVA) to Differentiate *Escherichia coli* O157:H7 Isolates

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Introduction: The identification of the dissemination routes of *Escherichia coli* O157:H7 on farms and in different food production systems is an important step in controlling this pathogen. It is often difficult to differentiate *E. coli* O157:H7 isolates, however, due to clonal similarity within the strain.

Purpose: Here we combined phage-typing with multi-locus variable number of tandem repeat analysis (MLVA) in attempt to develop a more powerful protocol for identifying specific O157:H7 isolates.

Methods: Forty-eight *E. coli* O157:H7 isolates from various sources (e.g., cattle manure, ground beef, human fecal samples, etc.) were analyzed by phage-typing and MLVA. MLVA was performed by comparing the number of tandem repeats at seven loci. For phage typing, 16 phages were isolated from wastewater treatment facilities throughout the State of Indiana. Five levels of lytic activity were determined based on plaque formation characteristics.

Results: MLVA was able to differentiate the isolates into 26 MLVA types. Phage-typing was able to differentiate the test isolates into 27 phage types. Importantly, phage-typing was able to differentiate isolates with the same MLVA-type from one another based on a combined MLVA- and phage-type.

Significance: Thus, these data indicate that combining these two techniques, both of which are rapid and require little labor, could result in a higher capacity to differentiate very similar *E. coli* O157:H7 isolates. Thus, they could be powerful tools in identifying critical sources of *E. coli* O157:H7 transmission in different food systems.

T6-05 Comparison of Real-Time RT-PCR and RT-LAMP Assays for Human Norovirus GII Detection Sensitivity

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Introduction: Loop-mediated isothermal amplification is a simple method increasingly researched for foodborne-pathogen testing/detection. RT-PCR and RT-LAMP assays have been reported to detect human noroviruses (hNoVs) in clinical samples.

Purpose: This study compared the detection sensitivity of previously described real-time RT-PCR to RT-LAMP assays using clinical outbreak samples.

Methods: Human norovirus GII clinical isolates (total of 9) were assayed by heat release or RNA extracts obtained by the TRIzol™ method using real-time SYBR Green I based RT-PCR kits and RT-LAMP assays in triplicate. Serially diluted heat-released samples were used in 50 µl reaction volumes. For the RT-PCR assay, reaction conditions included reverse transcription at 50 °C for 40 min, 94 °C for 3 min, and 42 cycles at 94 °C/45 s, 56 °C/45 s, 58 °C/30 s, and final extension at 72 °C/7 min, while the RT-LAMP assay was carried out at 63 °C for 90 min. Confirmation of product size (98 bp and Tm of 84 °C for RT-PCR) or ladder patterns for the RT-LAMP assay was obtained by agarose gel electrophoresis.

Results: The detection limit study indicated that viral RNA extracted by the TRIzol™ method consistently detected one-log RT-PCR unit (-1 dilution) using the real-time-RT-PCR assay for all 9 tested clinical samples. However, the detection limit improved by one log using the RT-LAMP assay compared to the RT-PCR assay. Heat-released samples showed one-log higher detection than RNA extracts by the RT-PCR assay (2-log RT-PCR units), but was less sensitive by RT-LAMP for some samples, due to stool sample inhibition.

Significance: The RT-LAMP assay can be completed within 1.5 h followed by agarose gel electrophoresis, without requiring expensive thermocyclers. The RT-LAMP assay is more sensitive than the RT-PCR assay for extracted RNA. This assay shows promise for the rapid screening of hNoV GII in clinical samples and has potential application for food testing.

T6-06 Attachment, Internalization and Dissemination of Human Norovirus Surrogates in Romaine Lettuce

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Developing Scientist Competitor

Introduction: Fresh produce is a major vehicle for transmission of human norovirus because they are easily contaminated both pre- and post-harvest. In fact, norovirus accounts for more than 40% of produce-associated foodborne illness. However, the attachment, internalization and dissemination of human norovirus in fresh produce are poorly understood. Internalized viruses would pose a significant risk to consumers because the viruses would be protected from all sanitation measures.

Purpose: The purpose of this research is to determine whether human norovirus surrogates can be internalized and disseminated in growing plant tissues.

Methods: Romaine lettuce was grown hydroponically in feed water containing two human norovirus surrogates (murine norovirus, MNV-1; and Tulane virus, TV). The hydroponic feed water was inoculated with either MNV-1 or TV to have a starting titer of 1×10^6 pfu/ml and plants were allowed to grow for 2 weeks. Leaves, shoots, and roots were homogenized and viral titers were determined by plaque assays at multiple time points throughout the study.

Results: We demonstrated that both MNV-1 and TV can be efficiently internalized and disseminated in Romaine lettuce grown hydroponically. Both viruses can attach tightly to roots and became internalized and disseminated to shoots and leaves at day 1 following virus inoculation. At day 3 post inoculation, the titer of MNV-1 in the shoots and leaves reached 3.3×10^4 pfu/g and 8.9×10^3 pfu/g, respectively. Similarly, the titer of TV in the shoots and leaves reached 2.8×10^5 pfu/g and 9.1×10^4 pfu/g, respectively, at day 3 post inoculation. The MNV-1 and TV viral titers detectable in the roots, shoots, and leaves increased gradually over 14 days to a final titer of 10^6 pfu/g.

Significance: These results indicate that human norovirus surrogates can be internalized in the roots and disseminated to shoot and leaf portions of the plant. A better understanding of the virus-fresh produce interaction would lead to the development of novel interventions to minimize virus contamination in fresh produce.

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T6-07 High Pressure Processing of Human Norovirus Virus-like Particles: Evidence That Human Norovirus May be Highly Pressure Resistant

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Developing Scientist Competitor

Introduction: High pressure processing (HPP) is a promising non-thermal technology for inactivating foodborne viruses; nevertheless, the effectiveness of HPP on inactivating human norovirus (HuNoV), the leading cause of acute gastroenteritis, is unknown because it cannot be propagated in cell culture. Developing a new model system, therefore, to understand the survival of HuNoV is urgently needed.

Purpose: The objective of this study was to evaluate the stability of HuNoV capsid to HPP using virus-like particles (VLPs) as a model.

Methods: HuNoV VLPs were treated at pressures ranging from 500 to 900 MPa at 4 °C for various holding times (between 1 and 60 min). HuNoV capsid disruption was evaluated by analyzing VLP structure integrity, and binding to its functional receptors, histo blood group antigens (HBGAs).

Results: Pressurization at 500-600 MPa, the pressure known to completely inactivate HuNoV surrogates (e.g., murine norovirus [MNV] and feline calicivirus [FCV]) in 2 min, was not sufficient to disrupt the structure and function of HuNoV VLPs, even with a holding time of 60 min. Disrupting efficacy of HPP increased with increasing pressures. Times required for complete disruption of HuNoV VLPs at 700, 800, and 900 MPa were 30, 10, and 2 min, respectively. Moreover, HuNoV VLPs were more resistant to HPP in their ability to bind type A than types B and O HBGAs. Additionally, the sub-VLPs (23-nm) appeared to be much more stable than the full size VLPs (38-nm).

Significance: While high levels of pressure (800-900 MPa) are required, a short holding time is capable of disrupting HuNoV VLPs. Our data suggest that HuNoV may be more resistant to HPP than MNV and FCV; therefore, the HuNoV VLPs may be a better model to study the survival of HuNoV under various conditions and further facilitate the use of HPP to inactivate pathogenic viruses, thereby improving the safety of high risk foods.

T6-08 Sensitivity of Murine Norovirus and Hepatitis A Virus to E-beam Irradiation in Whole Oyster and Oyster Homogenate

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Introduction: Shellfish are known to concentrate enteric viral and bacterial pathogens. Consumption of raw and uncooked oysters increases the risk of foodborne infections. Among the enteric viruses, noroviruses and hepatitis A virus (HAV) are of significance to the shellfish industry and to public health.

Purpose: To demonstrate the potential of E-beam irradiation processing as a pathogen kill-step to reduce or eliminate HAV and norovirus in shellfish.

Methods: Live oysters (*Crassostrea virginica*) were contaminated under simulated natural conditions with HAV and murine norovirus (MNV-1) in accumulation tanks. These whole oysters as well as oyster homogenates were subjected to E-beam irradiation at different doses ranging from 0.5 kGy to 5.5 kGy. The samples were processed to extract the viruses and plaque assays were performed for viral quantification using specific cell lines for MNV-1 (RAW 264.7) and HAV (FRhK-4), respectively. E-beam dose required to bring about 1-log reduction in the viral titer (D_{10} value) and percentage reduction in viral titer at different E-beam doses was determined for MNV-1 and HAV in both whole oyster samples and oyster homogenate.

Results: D_{10} values of MNV and HAV in oyster homogenate were 4.96 ± 0.6 kGy and 5.74 ± 0.8 kGy, which were not significantly different from that of the whole oysters (4.56 ± 1.8 and 5.6 ± 2.3 kGy, respectively). The D_{10} values of MNV-1 and HAV were high in both whole oyster and oyster homogenate indicating that oyster meat protects enteric viruses from ionizing radiation. However, at the current FDA approved maximum dose of 5.5 kGy, the use of E-beam irradiation of oyster homogenate will achieve a 91.2% and 92.5% reduction in MNV and HAV, respectively. In whole oysters, the reduction will be 90.3% and 86.7% for MNV and HAV, respectively.

Significance: The results suggest that E-beam inactivation at current FDA approved dose will result in a defined reduction of enteric viruses in oysters, which in turn can translate into reduction in shellfish-associated human illnesses.

T6-09 High-pressure Processing of Rotaviruses: The Roles of Strain Diversity and Treatment Temperature in Virus Inactivation

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Introduction: Rotavirus is an important cause of acute gastroenteritis in infants and children worldwide. Rotavirus is a double-stranded RNA virus that is characterized by substantial genetic diversity. Currently, five serotypes (G1–G4, G9) predominate, accounting for almost 95% of strains worldwide. High-pressure processing (HPP) is a promising non-thermal processing technology to inactivate foodborne viruses. However, whether HPP can effectively inactivate different rotavirus serotypes is not known.

Purpose: This work aims to compare the baro-sensitivity of different rotavirus strains to HPP and to gain a better understanding of the correlation between strain difference and pressure resistance.

Methods: Four different rotavirus serotypes G1 (Wa, Ku, and K8 human strains), G2 (S_2 human strain), G3 (SA-11 simian strain and YO human strain), and G4 (ST3 human strain) were treated at pressures ranging from 200 to 500 MPa at 4°C or 20°C for 2 min. The survival of rotavirus was quantified by plaque assay. The damage to viral structure and proteins was analyzed by electron microscope and SDS-PAGE, respectively.

Results: All rotavirus serotypes could be effectively inactivated (more than 5-log virus reduction) at pressure levels of 400-500 MPa for 2 min. Rotaviruses were more susceptible to HPP at 4°C compared to 20°C. Moreover, different strains of rotaviruses had different baro-sensitivity to HPP. Simian rotavirus SA-11 strain was more sensitive to HPP than human rotavirus strains. Furthermore, damage to virion structure by disruption of the viral capsid is the primary mechanism underlying HPP-induced viral inactivation. However, HPP did not degrade viral proteins or RNA.

Significance: Our results showed that (i) different rotavirus strains have different sensitivity to HPP; (ii) treatment temperature affects the effectiveness of viral inactivation; and (iii) damage of viral capsid is the primary mechanism underlying HPP inactivation of rotaviruses. This study also suggests HPP is a feasible technology to inactivate rotaviruses in food, water, and other fomites.

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T6-10 Internalization Rates and Survival of *Campylobacter jejuni* by *Acanthamoeba castellanii* Varies by Strains of *Campylobacter*

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Developing Scientist Competitor

Introduction: *Campylobacter jejuni* is one of the leading causes of foodborne illness in the U.S. Studies have shown that *C. jejuni* is able to survive phagocytosis by amoeba such as *Acanthamoeba castellanii* and reside intracellularly. Once inside of *A. castellanii, C. jejuni* has been shown to be protected from environmental factors including oxygen, pH, and chemicals (i.e., chlorine). This protection by amoebae may aid in the persistence of *C. jejuni* in the food industry.

Purpose: We investigated internalization rates among different *C. jejuni* strains into *A. castellanii* to determine if there is variation among *C. jejuni* strains.

Methods: *C. jejuni* strains were co-incubated 2 hours with *A. castellanii* to allow internalization. Washes with saline and gentamicin were used to remove extracellular and attached bacteria and Triton X-100 was used to lyse *A. castellanii* and release intracellular *C. jejuni*. Three trials were performed for each strain with triplicate samples per trial. Rates of internalization were determined by dividing the averages of total CFU *C. jejuni* recovered by total *A. castellanii* recovered.

Results: *C. jejuni* NCTC 11168 was employed as a reference strain for each experiment. *C. jejuni* strains 81-176 and NCTC 11168 Δ*flgK* showed a 2 log decrease in rate of internalization and 81-176 Δ*Cj0596* showed ~0.5 log increase in internalization into *A. castellanii* compared to the reference strain. Strains ATCC 43502 and 81116 showed a negative rate of internalization suggesting that these strains are not internalized or do not survive internalization.

Significance: The results of this study indicate that the rate of internalization (and therefore protection) of *C. jejuni* by *A. castellanii* is variable among strains. If internalization and protection from environmental stresses by *A. castellanii* is variable this may have implications for understanding survival of *Campylobacter* in poultry processing where amoebae have been isolated in previous studies.

T7-01 Exploring Temperature Patterns of Leafy Greens in Institutional Kitchens

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Introduction: Leafy greens have been linked to over 40 outbreaks of foodborne illness since 1990. As potential pathogens such as *Escherichia coli* O157:H7 can grow on or in cut leafy greens at temperatures above 5°C, the U.S. Food and Drug Administration recommends that leafy greens be kept at refrigerated temperatures (5°C or lower).

Purpose: There is a lack of research in determining the temperature profile of leafy greens within a food service establishment, especially the variation in temperatures through storage, preparation, serving, restorage, and reservice. The purpose of this study was to determine the temperature profiles and patterns that leafy greens in single serving clamshell containers were exposed to through a typical school meal program.

Methods: Temperatures of ready-to-eat leafy greens (whole head and cut/shredded) were recorded using data loggers, infrared and probe thermocouples in 20 schools over a 3-day period. Temperatures were taken by stabbing the probe into the leafy green, or through a thermocouple in the case of the data loggers; temperatures were taken in storage coolers, on the line, and in the prep cooler.

Results: Data showed that temperatures of leafy greens in clamshell containers reached above 5°C for more than 3 hours throughout the process as high as 8°C. Various temperature patterns were seen, including temperatures rising above 5°C for at least one hour up to 3 consecutive days. Leafy greens were shown in all cases to be exposed to temperature variability.

Significance: The results show the importance of developing a standard for thermometer use and temperature monitoring of leafy greens in school kitchens to avoid the potential for harmful pathogen growth. This data can be used both in risk assessment calculations and as an example to show food handlers the importance of a strict temperature verification regime.

T7-02 GAPs Training at University Farms, Orchards, and Gardens

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Introduction: Fruit and vegetable farmers are under pressure from buyers as well as impending produce safety regulation to understand and implement food safety practices such as Good Agricultural Practices (GAPs) on the farm to help insure fresh produce safety. Much of the training farmers receive is through university extension educators or researchers active in produce safety. Many universities have farms, orchards and garden plots where fresh produce is grown, harvested, and distributed for fresh consumption, but those involved in the production have not been trained or required to follow GAPs.

Purpose: Highlight and discuss approaches to correct the lack of GAPs training programs at universities that grow, harvest, and distribute fresh produce through multiple venues including commercial sale, student gardens, demonstration plots, and distribution of excess research crops.

Methods: A GAPs training program focused on worker training for university personnel was developed and delivered at Cornell University's New York State Agricultural Experiment Station. A special 2-hour train-the-trainer course was developed to help individual laboratory groups develop training programs for summer employees directly involved in fresh produce production.

Results: Implementation of GAPs at universities will have to be a step-by-step sustained commitment. University farms, orchards, and gardens are controlled by many different faculty members and program leaders making the implementation process very difficult and non-uniform. Successful implementation will require interest and support from university administration through farm labor at university research farms and student gardens.

Significance: There are three significant outcomes that result from addressing the lack of GAPs in university environments; setting a good example for practices actively promoted through extension programs, reducing risks to fresh produce grown, harvested and distributed at universities, and begin to meet fresh produce industry standards.

T7-03 Can a Passively Delivered Paper-based Educational Intervention Improve Restaurant Food Handler Knowledge?

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Introduction: Restaurants are a major reported source of the food that was eaten in foodborne outbreaks.

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Purpose: Using educational materials produced in English and Spanish in formats that included a straightforward brochure and an illustrated story-based style (comic book), this study determined the efficacy of this educational intervention when distributed in a passive manner.

Methods: From a total of 125 participating restaurants, 508 food handlers who spoke either English or Spanish were interviewed during January through July 2009 to determine baseline knowledge and identify which knowledge questions were most frequently answered incorrectly. An educational brochure and a comic book were created based on the most frequently identified knowledge gaps from the baseline knowledge study. Sixty-two restaurants (276 food handlers) were randomly assigned to the intervention group and 63 restaurants (232 food handlers) to the control group. A follow-up knowledge survey was then performed.

Results: There was an increase of 1.7 points (knowledge score rose from 67% to 73%) among the 35 food handlers that read either or both of the intervention materials compared to their baseline score ($P < 0.05$). For comparison, 101 control food handlers did not have a significant rise in their knowledge score. Knowledge in the intervention group about bloody diarrhea being a possible manifestation of eating ground meat that is not completely cooked increased by 32% ($P = 0.005$).

Significance: These data demonstrate that even with a passively delivered educational intervention, measurable, significant knowledge increases may be observed among restaurant food handlers. This study quantifies the need for more active approaches. Editing these materials and performing a more active approach that includes requiring food handlers to read the materials may lead to more substantial improvement in knowledge in a much larger number of food handlers and is an area for future research.

T7-04 Opening Markets: Identifying Barriers and Developing Guidance for GAP Certification

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Introduction: For small produce farmers, verification of good agricultural practices (GAPs) certification has been raised as a roadblock to entry into institutional markets. Currently there is a plethora of anecdotal information as to the technical and resource barriers associated with GAPs verification but there is a lack of actual data, collected in a structured manner, to confirm or refute concerns.

Purpose: The objective of this study was to observe current on-farm practices and cost of GAP implementation to better understand barriers, and direct strategies and resources to better enable market access.

Methods: Case studies were conducted using a combination of quantitative (costs and time calculations) and qualitative (in-depth interviews and self-diaries) collection methods with small fresh produce farmers (n=12) who were going through the process of attaining USDA GAPs certification. The farms, all less than 30 acres with multiple commodities were selected based on growing method, commodity diversity, labor, harvest seasons, and quantity of land. Preliminary onsite surveys were conducted to compare current practices to USDA GAP guidelines resulting in a risk practice score. The farms participated in monthly onsite interviews discussing on-farm practices, risk reduction strategies, GAPs requirements and economic impact.

Results: Survey results (n=12) demonstrated the lack of policy documentation (0%), traceability program (8%), and worker training (33%). Farmers expressed GAP implementation cost as an issue related to inadequate storage (83%), treatment of irrigation water (75%) and packaging (50%). Barriers identified in this study included interpretation of GAP principles, unclear buyer expectations, and site-specific risk recognition.

Significance: This case study was effective in identifying process and cost barriers to GAP certification, providing site-specific consultation, and determining parts of GAP certification not applicable to small farms. Onsite visits facilitated the development of resources to overcome barriers and strategies to mitigate the cost of implementation for small farms attempting GAP certification.

T7-05 Identifying Food Safety Risks for Minority Racial/Ethnic Consumers

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Developing Scientist Competitor

Introduction: Consumers represent the last line of defense against foodborne illness and it is estimated that a significant percentage of foodborne illnesses are caused by improper handling by consumers. Surveys have been an important tool to evaluate food safety knowledge and behaviors, but minority groups often lack representation among the entire sampling frame. These groups have distinct food cultures that may represent unique food safety risks, but remain unidentified due to small sample sizes or food handling questions which are not culturally relevant.

Purpose: This study attempted to identify potential food handling practices unique to cultural practices. A comparable proportion of Caucasian, African American, Asian, and Hispanic consumers were surveyed regarding their current food handling knowledge, practices, and food consumption patterns.

Methods: Phone surveys were administered in English, Spanish and Chinese, targeting primary meal preparers residing in Philadelphia, Pennsylvania, in the fall of 2011. The survey was based on the 2006 FDA and FSIS Consumer Food Safety survey with the addition of culturally themed questions derived from recent focus groups held with the target populations. Non-parametric survey analysis used SPSS 19.0.0.

Results: A total of 428 surveys were completed (25.5% Hispanic, 25.1% Caucasian, 24.8% African America, and 24.6% Asian). Caucasians were significantly ($P > 0.05$) less likely to purchase eggs at room temperature and purchase live poultry, compared to all minority groups. Minority consumers were more likely to ($P > 0.05$) prepare offals and body parts (chitlins, kidney, chicken liver, headcheese, chicken giblets, pork skin, feet, snout), not use a meat thermometer, and leave whole poultry in the oven overnight to cook compared to Caucasians.

Significance: The survey identified unique handling practices among minority groups, which may present an increased risk for foodborne illness. These groups may benefit from food safety education that is culturally appropriate, based on language and cultural food differences.

T7-06 Development of the "I'm Gloving It!" Campaign to Promote Glove Use Behaviors among College and University Dining Foodservice Workers

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Introduction: Every year, foodborne illness impacts thousands of individuals in the United States with most foodborne illnesses resulting from poor food handling practices in retail foodservice establishments. Proper hand hygiene is the simplest method of preventing the transmission of illness causing microorganisms onto food. Proper hand hygiene includes proper handwashing and glove use that comply with the guidelines specified in the FDA Food Code.

Purpose: The purpose of this study was to assess the effectiveness of a behavior change intervention to improve glove use behaviors among college and university dining foodservice workers.

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Methods: The "*I'm Gloving It!*" flyer that provided information about glove use in accordance with the *2005 Food Code*guidelines was developed. The term "I'm Gloving It!" was derived from the saying "I'm Loving It!" and serves as an innovative and creative way of educating younger foodservice workers about proper glove use. The flyer used visuals to convey the message of proper glove use in foodservice operations and a brief rationale of When? Why? and How?

A total of 64 hours of observational data was collected and behavior change was measured to assess the effectiveness of the glove use campaign (n = 32). Data was analyzed using SPSS 20.0, and descriptive statistics were calculated.

Results: It was found that glove use behaviors significantly improved among food handlers that received the proper glove use intervention. Non-compliance behavior among participants that did not receive any training was 66%, while non-compliance behaviors among participants that received training was 23%.

Significance: The visual tool was helpful in educating food handlers about proper glove use to help prevent foodborne illness and bring about change in food handling behaviors. Visual tools are a quick tool to educate foodservice workers of all generations, especially those belonging to the younger generation.

T7-07 Produce Safety Alliance – A Fresh Perspective on Produce Safety

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Introduction: The Produce Safety Alliance (PSA) was formed in 2010 through a cooperative agreement between Cornell University, the United States Department of Agriculture (USDA), and the Food and Drug Administration (FDA) with a focus on education before regulation. The PSA seeks to provide fundamental, science-based, on-farm food safety knowledge to fresh fruit and vegetable farmers, packers, and regulatory personnel while addressing future produce safety regulations resulting from the Food Safety Modernization Act (FSMA).

Purpose: The Produce Safety Alliance was created to develop a national fresh produce safety curriculum and training program for fresh produce farmers, federal regulators, and industry personnel to improve the understanding and implementation of food safety practices on the farm.

Methods: A diverse group of produce industry representatives including farmers participated in ten working committees (WCs) tasked with identifying critical challenges to implementation and effective risk reduction practices in the effort to help farmers establish meaningful on-farm food safety programs. WCs met on a monthly basis using conference calls for open discussion. All notes and recommendations were documented and made publically available through the PSA website.

Results: Since May of 2011, 60 meetings have been held with over 170 working committee members who have identified unique challenges and on-farm risks specific to small farms and evaluated current science recommendations to determine their impact on Good Agricultural Practices (GAPs) recommendations. Each WC developed recommendations and submitted final reports that will guide curriculum development and training delivery. The WCs established a network of individuals that will provide a conduit for the distribution, outreach, and engagement of a nationally-recognized produce safety training program.

Significance: A significant effort was focused on including small farm owners as well as a large portion of the fresh produce industry in the review of current GAPs recommendations. All fresh produce growers, regardless of size, need to be committed to understanding and implementing food safety practices since they produce food.

T7-08 Economic Benefits from a Food Safety Education Program

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Introduction: One of the functions of the Expanded Food and Nutrition Education Program (EFNEP) is to provide food safety education to low-income households through state extension programs. In Ohio, almost 3,500 households were served, with benefits accruing to over 7,000 household members. Before now, there has not been a study to determine the net benefits of EFNEP's food safety program in Ohio.

Purpose: The objective of this study is to ascertain whether the costs incurred by the food safety component of Ohio EFNEP are justified by its benefits.

Methods: The projected annual illness reduction from Ohio EFNEP food safety education is estimated based on measured behavioral changes, the risk profile of the population protected, discounted years of education effectiveness, and the effectiveness of behavioral change. The economic benefit of illness reduction due to this program is estimated using the enhanced cost of illness model and compared to program costs associated with food safety education.

Results: Preliminary results suggest that the Ohio EFNEP food safety program generates substantial net benefits. Annual benefits from the program amount to $3.1 million (90% C.I.: $0.5 to $8.2 million), in comparison to $300,000 in costs attributable to food safety education.

Significance: As budgets shrink food safety education programs are in danger of facing cuts. This study shows that food safety education provides benefits to Ohio that far exceed costs. The result is an improvement in both the health and well-being of Ohio residents.

T7-09 Effective Good Agricultural Practices Training for Farmers: A Two-day Approach

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Introduction: Fruit and vegetable farmers need to understand produce safety, risk assessment, the implementation of food safety practices, and how to develop a farm food safety plan in order to reduce contamination of fresh produce, stay competitive in the market place, and prepare for new produce safety regulations. Many farmers are not prepared to address on-farm produce safety issues or develop a farm food safety plan.

Purpose: Provide Good Agricultural Practices (GAPs) education and training for farmers so they can perform a risk assessment of their own farms, develop a farm food safety plan, and implement food safety practices such as GAPs to reduce identified risks.

Methods: A two-day GAPs training and food safety plan writing workshop was developed for fruit and vegetable farmers. The first day is focused on increasing produce safety knowledge and learning how to conduct a farm-wide risk assessment while the second day is focused on the farmers developing their own unique farm food safety plan. All workshops are evaluated for effectiveness and long-term follow up data is actively gathered.

Results: Since 2009, fourteen workshops with over 250 participants have been conducted throughout the state of New York. Ninety-nine percent of the participants would recommend the training to others and by the end of day two of the workshops, more than half the participants had completed over 50% of their farm food safety plans.

Significance: Fresh fruit and vegetable farmers are receiving training that is improving their food safety knowledge which allows them to perform on-farm risk assessments and develop farm food safety plans to address identified risks. This allows them to stay competitive in the market place while reducing food safety risks.

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T7-10 An Investigation of Attitudes and Behaviors Related to Food Safety Training in Chinese Restaurants in the U.S.: An Exploratory Study

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Developing Scientist Competitor

Introduction: As the number of foodborne illnesses associated with ethnic food appears to be increasing, ensuring food safety in ethnic restaurants is important for public health. Despite the large number of Chinese restaurants in the nation, limited research has been conducted to assess needs and challenges related to food safety training in Chinese restaurants.

Purpose: The purpose of this qualitative study was to explore variables influencing behaviors of U.S. Chinese restaurant owners/operators regarding the provision of food safety training for their employees.

Methods: In-depth interviews with owners/operators of independent, traditional, full-service Chinese restaurants in the U.S. were conducted. Contact information of various Chinese restaurant owners/operators was obtained from ChineseMenu.com. The interview questions were developed based on literature review, input from Chinese restaurant owners, and foodservice systems and food safety experts. A pilot study was conducted prior to data collection. All interviews were audio-recorded and transcribed verbatim before main themes were identified and categorized.

Results: Twenty Chinese restaurant owners/operators participated in interviews. Seventeen major Chinese cultural values (CCVs) were identified as affecting attitudes and behaviors related to food handling and food safety training at Chinese restaurants. Most participants felt satisfied with health inspections but several participants expressed the difficulty in following the health inspectors' instructions and understanding inspection reports. A few participants provided food safety training to their employees because it was required by state law. Lack of money, time, manpower, energy, and perceived needs for food safety training were recognized as major challenges for providing food safety training. Food safety training materials utilizing videos, case studies, and handbooks in Chinese were most preferred by Chinese restaurant owners/operators.

Significance: By understanding traditional CCVs, attitudes and behaviors related to food safety training, and preferred training methods, food safety educators may develop strategies to reach and influence Chinese restaurant owners/operators more effectively to improve food safety in Chinese restaurants.

T7-11 Produce Handlers' Handwashing Behaviors in Secondary School Foodservice Facilities

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Introduction: The importance of ensuring food safety in the nation's school foodservice operations where over 31 million students receive lunch daily cannot be overemphasized. Employee handwashing has been an ongoing challenge yet remains the most important behavior to ensure food safety, especially when handling fresh produce.

Purpose: The purpose of this study was to evaluate the produce handlers' handwashing behaviors in secondary school cafeterias through systematic observations.

Methods: The literature and expert advice guided development of the data collection instrument. Included was time and occasion of each observation when hands should have been washed, duration, areas of hands washed, and if recontamination occurred. The instrument was pilot-tested and revised before data collection by trained researchers at 14 secondary school cafeterias where fresh produce was served. The compute function was used to evaluate handwashing adequacy, and descriptive statistics were calculated to summarize the data.

Results: At 14 school cafeterias, 425 occasions where handwashing was necessary were observed. The most frequent occasions were "between changing tasks" (n=177) followed by "after touching equipment doors" (n=72), "before handling different food" (n=44), "before starting food preparation" (n=38), and "after cleaning equipment and utensils" (n=12). Of 425 occasions, employees washed hands on 128 occasions only (30.1%). Hand soap was used less often (n=95, 22.4%). In 44 cases, hands were recontaminated by touching faucets, clothes, and other equipment. Of those who used soap, the mean length of time for lathering was 4.3 seconds (max=16). In the majority of cases (n=60), participants only washed their palms and tops of their hands. Washing between fingers was observed only on 22 occasions.

Significance: Systematic observations revealed inadequate handwashing by produce handlers in school cafeterias. Given that no further heating will occur to kill pathogens and hands being the most frequent point of contamination, training on proper handwashing and motivation to comply may be necessary to ensure food safety.

T7-12 Assessment of Basic Food Safety Knowledge by Farmers Market Participants

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Introduction: Recent farmers market outbreaks of strawberries with *E. coli* O157:H7 in Oregon, cantaloupe with *Listeria monocytogenes* in Colorado, and guacamole, salsa, and tamales with *Salmonella*in Iowa have increased the concerns with safeness of products featured at farmers markets. Assessment of educational and resource gaps is essential to ensure that the participants in farmers markets are fully equipped to produce safe produces to consumers.

Purpose: The purpose of this experiment was to assess the current pre- and post-harvest food safety knowledge of Farmers Market participants in Iowa.

Methods: During two farmers market workshops given in Des Moines, Iowa, and Dubuque, Iowa, 68 farmers were asked to complete a survey that assessed their level of confidence with basic pre- and post-harvest food safety concepts (e.g., cross contamination, vectors of contamination, water/manure handling, temperature control, personnel, microbial testing, sanitation, food safety plan). The survey was scaled as none, low, moderate or high understanding of the specific concept and had blanks for the participants to provide examples for further assessment of understanding.

Results: Results from surveys indicate that there is a need to more food safety education to smaller farmers that participate in farmers markets. Participants had none to low understanding temperature control (n=41), presence of personnel training (n=52), microbial testing methods (n=62), and how to clean (n=42). Knowledge of microorganism of concern, vectors contamination and manure use has the highest scores for understanding, and knowledge of a food safety plan and product handling after harvest being split between moderate and low.

Significance: Food safety education to small and medium sized farmers that participate in farmers markets is critical to aid in the reduction of foodborne outbreaks in the U.S. By revealing educational gaps, extension programs can be modified to emphasize those areas of food safety education.

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T8-01 *Salmonella* in FSIS-tested Ready-to-Eat (RTE) Meat and Poultry Products, 2005-2011, with Special Reference to *Salmonella* in Pork Barbecue

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Introduction: *Salmonella* may be present in ready-to-eat (RTE) meat and poultry products, either through underprocessing or through cross-contamination from other raw (untreated) materials, contaminated products or surfaces, food handlers, or animal vectors. Thus, RTE meat and poultry products samples from the Food Safety and Inspection Service's (FSIS's) ALLRTE and RTE001 sampling projects are tested for *Salmonella* in addition to *Listeria monocytogenes*. ALLRTE is a random sampling project for all RTE product types, while RTE001 is a risk-based sampling project targeting post-lethality exposed RTE products.

Purpose: To evaluate 7 years of ALLRTE and RTE001 *Salmonella*test results.

Methods: FSIS analyzed results of *Salmonella*testing of RTE product samples collected under the ALLRTE and RTE001 sampling projects for calendar years 2005 through 2011. Samples were tested using FSIS Microbiology Laboratory Guidebook methods.

Results: There were 21,029 ALLRTE samples collected from 2,925 establishments and 58,991 RTE001 samples from 2,393 establishments in 2005-2011. This yielded 13 *Salmonella*-positive ALLRTE samples (overall average, 0.06%; annual range, 0- 0.13%) and 28 positive RTE001 samples (average 0.05%; range, 0.01%-0.09%), respectively. However, 6 of the 41 positive samples (14.6%) were obtained within a 40-day period in 2011. Over 60% of positive products were pork-derived and about 20% were from chicken. Four product types (sausage, pork barbecue, head cheese and multicomponent) accounted for over 60% of all positive samples. Interestingly, all 7 pork barbecue positives (17% of all positives) were from establishments that prepared their product using a vinegar- and pepper-based sauce. The pork itself was thoroughly cooked, but the sauce ingredients (added post-cooking) were not heated. In response to these positives, FSIS issued Notice 48-11 focusing on pork barbecue products.

Significance: Analysis of *Salmonella* data from the ALLRTE and RTE001 sampling projects has guided changes in policies, regulations, inspection procedures and enforcement actions relevant to preventing *Salmonella* contamination of RTE products.

T8-02 Differential Expression of *Salmonella* Tennessee Membrane-associated Genes in a Low Water Activity Food

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Developing Scientist Competitor

Introduction: Survival of *Salmonella* in low water activity foods has resulted in numerous product recalls and foodborne outbreaks. It is pertinent to determine the adaptation mechanisms utilized by *Salmonella* to survive in this adverse environment for prolonged periods of time. Once these adaptive mechanisms are defined, we can implement antimicrobials or processing conditions that are antagonistic to survival and reduce the likelihood of *Salmonella* contamination in these foods.

Purpose: The purpose of this study was to investigate the changes in gene expression profiles associated with fatty acid biosynthesis of *Salmonella*Tennessee exposed to a low water activity food (sugar).

Methods: Stationary phase *Salmonella* Tennessee (peanut butter outbreak strain K4643) was inoculated on sugar (treatment) or held in tryptic soy broth (TSB, control) and incubated for 1 h at 25°C. Total RNA was extracted using RNeasy Mini Purification Kits and the quantity, quality and purity of RNA was evaluated. This was followed by Real-Time Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) to compare gene expression profiles. The Relative Expression Software Tool (REST) was used to analyze gene expression using *rec*A and *ffh*as reference genes. The experiment was performed in triplicate.

Results: Genes involved in unsaturated (*fab*A) and saturated (*fab*D) fatty acid biosynthesis were observed to be significantly down-regulated 3.8- and 10.9-fold, respectively, when *Salmonella* Tennessee was held for 1 h in sugar ($P < 0.05$). The expression of cyclopropane fatty acid synthesis encoding gene *cfa* was found to be up-regulated 2.1-fold ($P < 0.05$). Expression of *fabB,* also involved in unsaturated fatty acid biosynthesis, was not significantly affected.

Significance: These results indicate that cyclopropane fatty acid alteration of the lipid membrane may be one mechanism by which *Salmonella* adapts to low water activity environments. Future research will determine if these/similar results are observed in other *Salmonella* serovars and if higher levels of cyclopropane fatty acids are found in phospholipids of *Salmonella* under similar situations.

T8-03 Effect of the rdar Morphotype on *Salmonella enterica* Dispersal by Rain and Aerosols

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Introduction: *Salmonella* has been associated with tomato outbreaks, and contamination has been traced to production areas. Mechanisms of *Salmonella*dispersal in the field are poorly understood.

Purpose: To assess the dispersal of *Salmonella*by rain onto tomato plants as affected by the presence of the rdar morphotype, trichome density, and post-rain aerosol formation.

Methods: GFP- kanamycin-resistant *Salmonella* Typhimurium MAE 110 (with rdar morphotype) or MAE 119 (without rdar morphotype) were dispensed in Petri dishes at 10^8 CFU/cm^2 in the center of a rain simulator. Rain intensities of 60 and 110 mm/h were applied for 5, 10, 20, and 30 min. For aerosol assessment, *Salmonella* was recovered from the air after rain at 0, 9.5, 28.5, 47.5, and 85.5 cm from the ground using an impinger containing lactose broth. Sixty-centimeter-tall tomato plants with fruits were placed inside the rain simulator immediately after the rain. For splash dispersal assessment, tomato plants varying in trichome densities were placed around the *Salmonella* source prior to the rain event. *Salmonella*cells were recovered from plants making leaf imprints on LB agar with kanamycin or by washing off the cells from leaf surfaces with peptone water.

Results: *Salmonella* recovery from the air after rain was mostly observed when cells expressing rdar (MAE 110) were used. Aerial *Salmonella* transferred to tomato fruits in plants following a distribution Beta (2.5950, 4.7393). Cells lacking rdar morphotype (MAE 119) showed a significantly higher dispersion than MAE 110 when the trichome density was below 200 per cm^2. Conversely, MAE 110 cells showed significantly higher dispersal at trichome densities above 300 per cm^{2}when compared to MAE 119.

Significance: After rain, *Salmonella* may form aerosols and transfer to tomato fruits on plants. *Salmonella* dispersal by rain splash is affected by the density of leaf trichomes and bacterial rdar morphotype.

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T8-04 Regional Risks for *Salmonella* spp., *Escherichia coli* O157:H7 and *Campylobacter* jejuni Contamination of Irrigation Pond Water in the Suwannee River Watershed

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Introduction: Outbreaks of human foodborne diseases associated with fresh produce have resulted in questions about the safety of irrigation water; however, associated risks have not been systematically evaluated.

Purpose: To investigate the water quality of vegetable irrigation ponds in the Suwannee River watershed and analyze the relationship between environment factors and the occurrence and distribution of the human pathogens *Salmonella*spp., *Escherichia coli* O157:H7 and *Campylobacter jejuni*.

Methods: Two water samples were collected from each of 10 vegetable irrigation ponds every month from January, 2011. Population densities and/or occurrence of *Salmonella*, *E. coli* O157:H7, *C. jejuni*and 22 environmental variables were measured for each pond. Denaturing gradient gel electrophoresis was conducted for the analysis of bacterial communities. Correlation coefficients were calculated to evaluate the correlations between the environmental factors and the population or occurrence of the three human pathogens. Partial least square analysis was used for discriminant analysis and generating a predictive model.

Results: All ponds were positive for *Salmonella* and *E. coli* O157:H7 mainly in summer and fall of 2011. *C. jejuni* was detected in 9 ponds, especially during spring. Temporal distribution of the pathogens differed among the ponds. Temperature was positively correlated to *Salmonella*, while dissolved oxygen concentration and oxidation-reduction potential (ORP) were negatively correlated. Presence of *Salmonella* was related to bacterial community composition. No correlations were found between *Salmonella* and fecal indicators. Populations of fecal coliforms, copiotrophic bacteria, and temperature were positively correlated to *E. coli* O157:H7 incidence, while the total nitrogen concentration (TN) and ORP were negatively correlated. TN and ORP were positively correlated to population and occurrence of *C. jejuni*. The occurrences of *E. coli* O157:H7 and *Salmonella*were positively correlated.

Significance: This research will provide a research-based comparison of indicator organisms and these human pathogens in a major fruit and vegetable growing area.

T8-05 Detecting *Salmonella* Enteritidis in Laying Hens and Eggs after Experimental Infection at Different Oral Dose Levels

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Introduction: The attribution of human illness to eggs contaminated with *Salmonella*Enteritidis (SE) has led to substantial commitments of resources (by both government and industry) to risk reduction and testing programs in egg-laying flocks. Cost-effective application of testing requires a thorough understanding of the outcomes of SE infections in hens.

Purpose: This study sought to resolve incompletely understood aspects of SE infections in laying hens which affect flock testing efforts, including relationships between quantitative oral exposure levels and important detectable parameters: the frequency and duration of fecal shedding, the frequency and magnitude of internal organ colonization and the frequency of deposition inside developing eggs.

Methods: In six trials, groups of specific-pathogen-free laying hens were experimentally infected with oral doses of 10^4, 10^6 or 10^8 CFU of phage type 4 or 13a SE. Fecal shedding was monitored for 8 wk post-inoculation (PI), the frequency and concentration of SE cells in livers were determined at 1 and 3 wk PI, and eggs were collected for 4 wk PI and cultured for SE contamination in yolk and albumen.

Results: Fecal shedding of SE declined over time and was last detected in the 10^4 CFU dose group at 3 wk PI. At 4 wk PI, 6% of hens in the 10^6 CFU dose group and 28% of hens in the 10^8 CFU dose group shed SE in feces; a few birds in these groups were still shedding at 8 wk. SE was isolated from 65% of livers from hens given 10^8 CFU but from only 15% of hens given 10^4 CFU, and the highest inoculation level was likewise associated with significantly ($P < 0.05$) higher SE concentrations in livers. Increasing inoculation doses were associated with both significantly ($P < 0.05$) more frequent egg contamination and a significantly ($P < 0.05$) higher propensity toward deposition in albumen.

Significance: These results demonstrate that the oral exposure dose has significant effects on important detectable parameters of SE infection in laying hens which could potentially influence testing outcomes.

T8-06 Geographical Factors Influence the Spatio-temporal Distribution of *Listeria monocytogenes* in Natural Environments of New York State

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Developing Scientist Competitor

Introduction: Agricultural lands in New York State (NYS) and other agricultural producing regions are widely interspersed with areas of natural land cover (e.g., forests, wetlands), which may serve as environmental reservoirs of foodborne pathogens such as *Listeria monocytogenes*. However, there is limited data regarding the geographical factors that influence the spatial and temporal distribution of *L. monocytogenes*in natural environments; hence, the risk these areas pose to agriculture remains poorly understood.

Purpose: The purpose of this study was to utilize meteorological and landscape data to identify potential reservoirs of *L. monocytogenes*.

Methods: Samples from natural environments (e.g., wildlife management areas and national forests) in NYS were analysed for the presence of *L. monocytogenes*. Classification trees were constructed using meteorological, topological, and soil properties as predictors.

Results: *L. monocytogenes* was detected in 8.8% (65/735) of samples. Classification trees provided clear rules for dividing sites according to presence or absence of *L. monocytogenes*. Meteorological variables served as global predictors of*L. monocytogenes* presence. *L. monocytogenes* presence was more likely during the summer and fall seasons when minimum temperatures did not exceed 13 °C, and when there were less than three freeze-thaw cycles in the eight days prior to sampling. When the above conditions were met, *L. monocytogenes* was more likely found in eastern NYS than central NYS. Alternatively, a surrogate to this rule showed that samples less than 169 m from open water sources were more likely positive for *L. monocytogenes*.

Significance: These data contribute to understanding the influence of geographical factors on the spatial and temporal distribution of *L. monocytogenes*. The high prevalence of *L. monocytogenes* suggests that this pathogen can be introduced from natural environments to food-associated environments, and these data will be used to gain further insight into the environmental transmission of *L. monocytogenes*.

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T8-07 Antibiotic Resistance and Genetic Diversity of *Listeria* spp. Not Showing 16S rDNA Sequence Similar to Known *Listeria* Strains Isolated from Pekin Ducks and their Environmental Sample

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Developing Scientist Competitor

Introduction: *Listeria* spp., particularly *L. monocytogenes,* are responsible for rare foodborne infections albeit very high mortality especially in immune-challenged individuals. The isolation of strains of *Listeria*not showing 16S rDNA sequence similarity to known strains highlights a possible new risk and potential for the emergence of new foodborne pathogens resulting from human activity. Resistance of foodborne pathogens to multiple antibiotics is of concern worldwide and makes it difficult to treat infected patients when needed, while molecular typing methods provide useful information for tracing the source of infection.

Purpose: The objectives of this study was to determine the antibiotic resistance, presence of plasmids and hemolysin gene and to characterize new *Listeria*strains isolated from ducks and their environmental sample using RAPD, REP and ERIC to determine their genetic relatedness.

Methods: A total of 531 ducks and their environmental samples were examined between 2009 and 2010 of which 5 were positive for unknown *Listeria* spp. These five positive *Listeria* spp. not showing DNA homology with known species, confirmed by sequencing at Eurofins MWG, were examined for their resistance to 15 antimicrobial agents using the disc diffusion method. They were also examined for the presence of plasmids (using Promega wizard® plus minipreps DNA purification system by following manufacturer's instructions). Detection of the *hlyA*gene, ERIC, REP and RAPD typing were done following procedures published in peer-revewed journals.

Results: 0.94% (5/531) of ducks and duck environmental samples examined in Penang, Malaysia between 2009 and 2010 were positive for a potential novel *Listeria* sp. They were isolated from fecal swabs (3 isolates), cloacal swab (1 isolate) and pond water (1 isolate). The five isolates were all resistant to cefotaxime, nalidixic acid and tetracycline but susceptible to ampicillin and nitrofurantoin. Four out of five of the isolates were also resistant to cephalothin, gentamicin and streptomycin. MAR index ranged from 0.2 to 0.8. All the isolates harbored plasmids and the *hlyA*virulence-associated gene. Plasmid sizes ranged from 2.1-24 kb. Each genotyping method (RAPD, REP, and ERIC) grouped the isolates into four similar types. Typing results indicated greater genetic diversity among the 5 isolates.

Significance: Our results suggest that these *Listeria* isolates from ducks may be pathogenic due to the presence of the *hlyA* virulence gene and its multi-antibiotic resistance profile. It is possible that these represent a novel species. This report also draws attention to the emerging importance of this foodborne pathogen and to the need for monitoring changes in antibiotic resistance patterns so that efficient risk and control management strategies can be developed.

T8-08 *Listeria monocytogenes* Strains Differ in Their Ability to Form Biofilms but Show Similar Swarming and Response to Sanitizers

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* is composed of two subpopulations that differ in their likelihood and ability to cause invasive listeriosis. Epidemic clone (EC) strains have been linked to most listeriosis outbreaks and sporadic cases in several countries, yet are infrequently isolated from foods. Nearly 50% of *L. monocytogenes* isolates from foods and 40% of isolates from food-associated environments carry one of 18 described mutations leading to a premature stop codon (PMSC) in the key virulence factor Internalin A (InlA, encoded by *inlA*).

Purpose: The purpose of this study was the evaluate the hypothesis that *L. monocytogenes* isolates carrying a PMSC mutation in i*nlA*adapted to food-associated environments as a trade-off for loss of full virulence.

Methods: We assembled 13 EC strains and 15 strains carrying the most common PMSCs and tested the ability of these strains to swarm, form biofilms, and survive recommended and sub-inhibitory concentrations of two sanitizers. Swarming assays were performed at 30 °C in semi-soft agar. Biofilm experiments were performed under biofilm promoting conditions in polyvinyl chloride microplates, followed by crystal violet staining to quantify biofilm formation. Lastly, broth microdilution assays determined the susceptibility of strains to recommended and sub-inhibitory concentrations of multi-quaternary ammonium compound (QAC) and sodium hypochlorite (SHC) under nutrient rich and nutrient limiting conditions.

Results: EC and *inlA* PMSC strains did not differ in their ability to swarm after 72 h ($P > 0.05$). *inlA* PMSC strains demonstrated enhanced biofilm formation compared to EC strains, after 3 and 5 days ($P = 0.0199$ and $P = 0.0068$, respectively). Under nutrient rich conditions, all but one strain were found to be susceptible to all concentrations of QAC and all but three strains were resistant to all concentrations of SHC. However, under nutrient limiting conditions, all strains were susceptible to all concentrations of QAC and some concentrations of SHC.

Significance: *inlA* PMSC strains demonstrated increased biofilm formation when compared to EC strains, which may explain their common presence in food-associated environments. EC strains showed similar swarming abilities when compared to *inlA* PMSC strains and both subpopulations showed similar responses to sanitizers.

T8-09 Analysis of Data from FSIS Routine and Intensified Sampling Programs for *Listeria monocytogenes* from Establishments that Produce Ready-to-Eat Products

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Introduction: The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) samples ready-to-eat (RTE) meat and poultry products and processing environments for *Listeria monocytogenes* (*Lm*) through routine sampling (RLm) and intensified verification testing (IVT) programs. RLm sampling is performed routinely at RTE establishments, while IVT is performed in response to positive results from routine testing programs. A Food Safety Assessment (FSA) is performed along with RLm and IVT to evaluate food-safety controls at the establishment.

Purpose: To analyze results from FSIS RLm and IVT sampling programs and to identify possible trends in the data.

Methods: Samples were collected proportional to establishment size (RLm) or based on investigative needs (IVT). Products were collected in the final packaged form, and food contact surface (FCS) and non-FCS (NFCS) samples were collected using sterile SpongeSicles®hydrated with Dey-Engley (DE) broth. Samples were analyzed using methods in the FSIS Microbiology Laboratory Guidebook.

Results: From 2009–2011, 267/31,511 (0.85%) of RLm samples and 199/9,231 (2.16%) of IVT samples were positive. For RLm, 10/5,170 (0.19%) of product, 64/17,733 (0.36%) of FCS and 193/8,608 (2.24%) of NFCS samples tested positive. For IVT, 17/1,392 (1.22%) of product,

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75/5,034 (1.49%) of FCS, and 107/2805 (3.81%) of NFCS tested positive. In previous years (2005–2008), 135/16,284 (0.83%) of RLm samples and 486/13,241 (3.67%) of IVT samples were positive.

Significance: From 2009-2011, positive samples were highest for the IVT program, likely because an IVT is triggerd by routine positives, indicating possible sanitation issues. For RLm and IVT programs, positives were highest for NFCS, followed by FCS, and product samples, an expected finding because *Lm* is an environmental contaminant. Compared to previous years (2005-2008), positives increased slightly for RLm and decreased for IVT. FSIS uses results from IVT and RLm along with FSAs to identify food-safety risks in establishments and to help protect public health.

T8-10 Comparison of Growth of a Combined Strain *Listeria monocytogenes* Challenge Study Inoculum in Different Chloride Salt Solutions

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Introduction: Reduced sodium food formulations are a current focus of product development and redevelopment. However, reduction of sodium in foods that are time and temperature controlled for safety may impact the growth of psychrotrophic bacterial pathogens, such as *Listeria monocytogenes*. Potassium chloride (KCl) is the most popular choice as a replacement for NaCl, but comparisons of these and other chloride salts against growth of *L. monocytogenes* are needed.

Purpose: This study compared growth of *L. monocytogenes*in broth at equal chloride ion concentrations achieved using five different chloride salts and at unequal chloride ion concentrations made with 2% of each of the same five salts

Methods: A five-strain culture of *L. monocytogenes* was inoculated at ca. 3 log CFU/ml into tryptic soy broth with 0.4% yeast extract (TSBYE) supplemented with $CaCl_2$, KCl, LiCl, $MgCl_2$, or NaCl at chloride equivalent concentrations standardized to 2% (w/v) NaCl, or in TSBYE with the same salts at a 2% (w/v) concentration. Broths were incubated at 35 °C and populations were enumerated after 48 h by plating on modified Oxford medium (MOX) and tryptic soy agar with yeast extract (TSAYE). Average log populations were compared with repeated measures ANOVA and Tukey's test.

Results: As determined by plating on TSAYE, populations of *L. monocytogenes* in equal chloride ion concentrations increased by 6.13, 5.86, 5.83, 5.56, and 5.22 log, for NaCl, $MgCl_2$, KCl, LiCl, and $CaCl_2$, respectively. However, counts on MOX were significantly ($P < 0.05$) lower than TSAYE counts for NaCl, $MgCl_2$, and $CaCl_2$, indicating possible cellular stress. TSAYE counts showed that LiCl at 2% (w/v) was significantly more suppressive to *L. monocytogenes* growth compared to other salts, allowing only a 5.19-log increase while NaCl and KCl permitted 6.21- and 6.18-log increases, respectively. At 2%, $CaCl_2$similarly caused the greatest difference between TSAYE and MOX plate counts.

Significance: The enhanced inhibition and apparent stress of *L. monocytogenes* caused by growing in the presence of $CaCl_2$ compared to the chloride equivalents of other salts should be explored further. This work provides the foundation for further research on inhibition of the pathogen by chloride salts in combinations with other antimicrobials in food systems.

T8-11 Desiccation Survival of *Listeria monocytogenes* in Mixed Biofilms with *Pseudomonas fluorescens*, *Serratia liquefaciens* and *Shewanella putrefaciens*

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes*has been found to withstand harsh environmental conditions including desiccation. The non-linear bacterial desiccation inactivation kinetics results in a significant tail and the resistant sub-population has been shown to resume growth once rehydrated. The pathogen is known to form biofilm when in co-culture with other bacteria found in food products.

Purpose: The objective of this study was to investigate the desiccation survival of *L. monocytogenes* in mixed biofilms with *P. fluorescens*, *S. liquefaciens* and *Sh. putrefaciens*.

Methods: Cultures of *L. monocytogenes* 568 (serotype 1/2 a), *Sh. putrefaciens* A2, *P. fluorescens* ATCC 11105 or *S. liquefaciens* 2RT were prepared in Tryptic Soy Broth (TSB) +1% glucose, adjusted to 10^4 CFU/ml and deposited on stainless steel (SS) coupons to form mono- or binary-species biofilms with *L. monocytogenes* and the Gram-negative bacteria (48 h, 15°C & 100% RH). The coupons were subsequently desiccated (43% RH, 21 days at 15°C). To determine the kinetics of desiccation survival, selective agars (BHI, Oxford, Iron agar with PenicillinG, *Pseudomonas*CFC agar and VRBG) were used to enumerate survivors. The resulting survivor curves were fitted to the double Weibull model. Biofilms were additionally characterized by Scanning Electron Microscopy.

Results: The presence of the Gram-negative spoilage bacteria with the exception of *Sh. putrefaciens* reduced ($P < 0.05$) the number of *L. monocytogenes* cells on the SS coupons, indicating that competition decreased its growth and/or biofilm formation. However, the survival kinetics of *L. monocytogenes* in dual-species biofilms was not ($P > 0.05$) different from that in the mono-species biofilm, meaning that the competitor species did not alter desiccation survival of *L. monocytogenes*cells. Microscopy revealed different biofilm forming capabilities in the mono- and binary bacterial combinations.

Significance: The presence of competitor bacteria may significantly decrease the initial level of *L. monocytogenes* populations but they do not affect the desiccation resistance of the pathogen.

T8-12 Transcriptional Profile of *Listeria monocytogenes* Exposed to Sublethal Chlorine Dioxide

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Developing Scientist Competitor

Introduction: There is increasing interest in the use of chlorine dioxide (ClO_2) as a sanitizer in food systems. ClO_2 is a strong oxidizing sanitizer that effectively decreases contamination of foodborne microorganisms on the surfaces of foods and food processing equipment. To date, little is known how ClO_2 inactivates microorganisms and the microbial stress response mechanisms foodborne pathogens employ to survive this sanitizer.

Purpose: The purpose of this study was to identify genes differentially expressed in *Listeria monocytogenes* exposed to sublethal ClO_2 concentrations to gain insight into stress response mechanisms that may be used to survive ClO_2exposure.

Methods: *L. monocytogenes* 10403S was grown to early log phase (OD_{600} = 0.4) in Brain Heart Infusion (BHI) broth and exposed to 300 mg/l of aqueous ClO_2 at 37°C, 230 rpm. RNA was extracted following exposure to ClO_2; gene expression levels from untreated and treated cells

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were compared on 70-mer *L. monocytogenes* microarrays. Expression fold changes ≥ 1.5 with a corresponding adjusted *P*-value of <0.05 were considered significantly differentially expressed. RNA extractions and microarrays were completed in quadruplicate.

Results: Cells treated with 300 mg/l aqueous ClO_2 for 15 minutes resulted in a 0.58 + 0.24 log CFU/ml reduction of *L. monocytogenes*. Data analysis identified 238 up-regulated and 116 down-regulated genes in *L. monocytogenes* 10403S exposed to ClO_2. Among the 238 up-regulated genes, 115 genes have been previously reported to be regulated by σB, a major stress response regulator. Five up-regulated genes encoding proteins with oxidoreductase activity were identified, which may significantly contribute to ClO_2stress survival.

Significance: This is the first transcriptomic study, to our knowledge, to investigate stress response of *L. monocytogenes* to sublethal concentrations of ClO_2. Understanding potential survival mechanisms may provide significant insight into the specific mode of action of ClO_2 as a sanitizer.

T9-01 Temperature and Time-dependence Effects of Cranberry Proanthocyanidins and Pomegranate Polyphenols on Hepatitis A Virus Infectivity

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Introduction: Cranberry proanthocyanidins (C-PAC) and pomegranate polyphenols (PP) are known for their antimicrobial activities against bacteria and also enveloped and non-enveloped viruses. However, their effects on hepatitis A virus (HAV) have not yet been extensively studied.

Purpose: The effects of C-PAC and PP on the infectivity of HAV after exposure from 0 to 1 h were evaluated at ambient temperature and 37°C.

Methods: HAV at ~5 log PFU/ml was mixed with equal volumes of C-PAC at 0.15 mg/ml, PP at 4 mg/ml, or water and incubated for up to 1 h at room temperature or 37°C. Viral infectivity after triplicate treatments was evaluated using standardized plaque assays in duplicate.

Results: The anti-HAV effects of C-PAC and PP were found to be time-dependent. At 37°C, 0.15 mg/ml C-PAC caused an instant HAV reduction of 0.4 log PFU/ml upon mixing and a 1.4 log PFU/ml reduction within the next 10 min of treatment, resulting in a total titer reduction of ~1.9 log PFU/ml within 1 h. At 37°C, no titer reduction of HAV was observed upon immediate mixing of the virus with 4 mg/ml PP, but caused a reduction of 0.4 log PFU/ml within 10 min of treatment, resulting in a total ~1 log PFU/ml reduction within 1 h. Thus, 0.15 mg/ml C-PAC was found to be more effective in HAV reduction than 4 mg/ml PP at 37°C. Comparable reduction of HAV was obtained with 4 mg/ml PP at room temperature and 37°C. However, the anti-HAV effect of C-PAC was found to be temperature-dependent with significantly lesser reduction at room temperature ($P < 0.05$).

Significance: These results show that C-PAC and PP appear to have promise as natural antivirals for HAV reduction and give an idea of the time-dependence mechanism of action.

T9-02 Genes Involved in Biosynthesis of Paenibacillin, a Novel Antimicrobial Peptide

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Developing Scientist Competitor

Introduction: Lantibiotics are ribosomally synthesized lanthionine-containing antimicrobial peptides produced by Gram-positive bacteria. Paenibacillin, a lantibiotic from *Paenibacillus polymyxa* OSY-DF with an unusual N-terminal acetyl group, shows potent activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, *Listeria monocytogenes* and *Mycobacterium smegmatis*.

Purpose: This study describes the biosynthesis of paenibacillin and its N-terminal acetylation.

Methods: The structural gene of paenibacillin was identified by polymerase chain reaction; the complete biosynthetic gene cluster was determined by whole-genome sequencing using Illumina's next generation technology. *De novo* assembly of the *P. polymyxa* OSY-DF draft genome was performed using CLC Genomics Workbench software.

Results: In the bacterial genome, a 12.3-kb DNA fragment, consisting of 11 open reading frames (ORFs), is involved in paenibacillin production, modification, immunity, regulation and transportation. One of the ORFs (*paenN*), which encodes a protein with high sequence similarity to F-pilin acetylase, may be responsible for the N-terminal acetylation of alanine during paenibacillin biosynthesis. Unlike other lantibiotics, an *agr*-like quorum sensing system encoded by the biosynthetic gene cluster may regulate the production of paenibacillin in *P. polymyxa* OSY-DF.

Significance: These findings elucidate the pathway of paenibacillin biosynthesis and pave the way to produce paenibacillin variants through genetic manipulation. The study also reports a putative acetylase responsible for peptide N-terminal acetylation, which is extremely rare in bacteria.

T9-03 Optimal Dispersion of nanoZnO and Antimicrobial Activity against *Staphylococcus aureus* and *Escherichia coli*

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Introduction: Bacterial contamination is of great concern to the food industry. Inorganic compounds in nanosize present antibacterial activity at low concentrations because of their high surface area to volume ratio. NanoZnO synthesis methods allow the production of separated nanoparticles. However, single primary nanoparticles tend to form particle collectives which may result in loss of antimicrobial activity.

Purpose: To optimize nanoZnO dispersion with response surface methodology (RSM) and test nanoZnO antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Methods: The effects of (1) power, (2) probe sonication time and (3) the presence of the dispersant agent ($Na_4P_2O_7$) on nanoZnO size were investigated using a 2^3 full factorial design. To study the time and power of sonication effects over an extensive range, a central composite design (CCD) was also applied. The optimized dispersion condition was chosen to obtain lowest nanoZnO size. Concentrations of nanoZnO (0, 1, 5 and 10%) were tested by the agar well diffusion method.

Results: The lowest nanoZnO size (259 nm) was obtained at conditions of 50 min, 200 W, in the presence of $Na_4P_2O_7$, whereas the highest nanoZNO size (506 nm) was obtained at conditions of 20 min, 400 W, in the absence of $Na_4P_2O_7$. The RSM showed the lowest nanoZNO size (238 nm) with optimal conditions at 200 W, 45 min of sonication in the presence of $Na_4P_2O_7$. At 1% and 5%, nanoZNO presented the same inhibition zone (1.0 cm) against *S. aureus*, with this zone increasing to a 1.5 cm for 10% NanoZNO. NanoZnO at 1% had no effect against *E. coli,* whereas 5 and 10% nanoZnO created inhibition zones of 1.5 and 1.8 cm, respectively.

Significance: Optimal nanoZnO dispersion presented antimicrobial activity against foodborne pathogens. NanoZnO is a promising antimicrobial for food preservation with applications for incorporation in polymers intended as food contact surfaces.

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T9-04 Time-dependent Effects of Myricetin and L-epicatechin against Human Norovirus Surrogates

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Introduction: Plant-derived polyphenols, myricetin and L-epicatechin, are known to have antimicrobial properties and health benefits. Their antiviral activities have been demonstrated against several viruses including human immunodeficiency virus-1, herpes simplex virus, influenza A virus, and human rotavirus. Studies to understand their effect against human noroviruses are needed.

Purpose: This study determined the time-dependent antiviral effects of myricetin and L-epicatechin at two concentrations on foodborne norovirus surrogate infectivity at 37˚C.

Methods: The lab-culturable surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1) at titers of ~5 log PFU/ml were each individually mixed with equal volumes of myricetin or L-epicatechin at 0.5 and 1 mM for up to 2 h at 37°C. Treatments were neutralized in cell culture medium containing 10% fetal bovine serum, followed by serial dilution and plaque assays in duplicate and repeated thrice.

Results: FCV-F9 was found to be reduced to undetectable levels by myricetin at both concentrations, and decreased by 1.1 and 0.3 log PFU/ml with L-epicatechin at 0.5 and 0.25 mM (final concentration), respectively after 2 h. MNV-1 showed no significant titer reduction by myricetin or L-epicatechin at the two tested concentrations over 2 h. The antiviral effects of myricetin and L-epicatechin on FCV-F9 were found to be time-dependent. FCV-F9 was reduced by ~1.8 log PFU/ml upon immediate mixing with equal volume of 1 mM myricetin, further decreased by ~1.3 log PFU/ml within 1 h and to undetectable levels after 1.5 h at 37°C. In comparison to 0.5mM myricetin, L-epicatechin at 0.5mM decreased FCV-F9 titers by 0.3 log PFU/ml within 1 h and by ~ 1 log PFU/ml after 2 h at 37°C.

Significance: Our results indicate that the two plant-derived polyphenols at the tested concentrations appear to be effective only against FCV-F9 over 2 h at 37˚C and not against MNV-1. Further studies using higher concentrations and longer incubation times may be necessary to determine any improved anti-noroviral effects and to understand their mechanism of action.

T9-05 Screening Antimicrobial Activities of the Herbal Plants against *Listeria monocytogenes* and Cytotoxicity Assays of the Plants Using Caco-2 Cell

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I***ntroduction:*** Although various synthetic antimicrobials have been used to control *Listeria monocytogenes* in foods, therapeutic herbal plants could be considered as antimicrobial alternatives because of consumers' demand.

Purpose: This study evaluated the antimicrobial activities of therapeutic herbal plants on *L. monocytogenes* and their cytotoxicity on Caco-2 cell.

Methods: The paper disks impregnated with 69 herbal plants were placed on brain heart infusion agar, where *L. monocytogenes* strains were overlaid. After incubation (37°C, 24 h), clear zone diameters were measured to select the herbal extracts showing antilisterial activity. *L. monocytogenes* strains were inoculated in 96-well plates containing 100 ¥ìl of two-fold serial diluents of the selected herbal extracts, followed by incubation (37 °C, 24 h) to determine MICs (minimal inhibition concentration). Moreover, aqueous portions of the wells that displayed no growth were spread-plated on BHI agar plates, and the plates were incubated at 37°C for 24 h to determine MBCs (minimal bactericidal concentration). The stability of the selected extracts were also examined to food-related conditions such as heat (60-100°C), NaCl (0-6%), and pH (4-7). In addition, two-serial dilutions of the selected herbal extracts were added to the wells containing Caco-2 cells and incubated at 37°C for 2 days in 5% CO_2 atmosphere to evaluate cytotoxicity of the herbal extracts.

Results: Of 69 herbal extracts, *Psoraleae semen* L. (Bogolji) and *Sophorae radix* L. (Gosam) extracts showed more than 10 mm of clear zone diameter, and their MICs were 8-16 µg/ml and 128-256 µg/ml depending on strains for Bogolji and Gosam, respectively. The MBCs were also 16 (Bogolji) and 256 µg/ml (Gosam). Heating, NaCl and acidic condition did not affect ($P > 0.05$) the antilisterial activity of Bogolji and Gosam. Cytotoxic activities were observed only at high concentration (128 µg/ml) of Bogolji extract.

Significance: The results indicate that Bogolji and Gosam could be used as potential phytochemicals to control *L. monocytogenes*.

T9-06 Chemical Decontamination of Footwear Soles to Limit Microbial Transfer in a Dry Environment

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Introduction: Safety of foods is often dependent on Good Manufacturing Practices (GMPs) including maintenance of manufacturing environments that prevent contamination of in-process or finished product materials. Decontamination of footwear soles by chemical sanitizers is often used to control the ingress and spread of pathogenic microorganisms over floor surfaces, although little has been published to validate effectiveness.

Purpose: This study evaluated four decontamination treatments for efficacy in reducing microbial populations on footwear soles and for reducing transfer from soles to floors.

Methods: Footwear soles were inoculated with an equal-part mixture of *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Serratia marcescens*, donned, and subjected to treatment with aqueous quaternary ammonium sanitizer (Aqueous QAC, 1,000 ppm) in a footbath, dry quaternary ammonium sanitizer (Dry QAC, 1.2% (wt/wt)) in a footbath, 58.6% isopropyl alcohol solution containing 200 ppm quaternary ammonium compounds (IPA QAC) sprayed onto footwear soles and an IPA QAC spray followed by Dry QAC in a footbath (IPA QAC/Dry QAC). Before and after treatment, footwear soles and floor surfaces were sampled to determine microbial levels. Means of recovered populations based on results of three replicate experiments were calculated and compared using ANOVA.

Results: No significant differences ($P > 0.05$) in populations on soles were observed upon treatment with Aqueous QAC, Dry QAC, and no treatment (control). Decontamination with IPA QAC and IPA QAC/Dry QAC resulted in significant ($P < 0.05$) 2.3 and 3.5-log reductions, respectively. Populations recovered from floor surfaces after IPA QAC and IPA QAC/Dry QAC (2.14 and 1.98 log CFU, respectively) were significantly lower ($P < 0.05$) than those recovered prior to treatment (3.11 mean log CFU), whereas transfer was not impeded by Aqueous QAC or Dry QAC alone.

Significance: Results of this study demonstrate that use of IPA QAC in the decontamination of footwear may provide an effective barrier against the spread of microorganisms by foot traffic. Furthemore, use of IPA QAC spray may be more effective than Aqueous QAC, 1,000 ppm in a footbath for decontamination of footwear, while helping to reduce presence of water in manufacturing plant environments.

T9-07 Mechanisms of the Resistance of *Bacillus subtilis* Spores to Pulsed UV-Light

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Introduction: Mechanisms involved in bacterial spore resistance to Pulsed UV-Light (PL) are poorly documented in contrast to mechanisms of UV-C resistance. Pulsed UV-Light consists in short time (< 1 ms) - high energy light flashes of broad spectrum (200 -1100 nm) and rich in UV. The UV-C wavelengths are essential for the bactericidal activity.

Purpose: Factors that play a major role in PL resistance were compared to those implicated in resistance to UV-C continuous radiation.

Methods: Ten *B. subtilis* strains carrying mutations of the 168 parental (Wt) strain in genes coding for proteins involved in spore resistance to UV were used to examine the relative contributions of those proteins and related mechanisms in spore resistance to PL. Spores were exposed to PL fluences comprised between 320 and 1770 mJ/cm^2 and continuous UV-C doses comprised between 25 and 150 mJ/cm^2.

Results: Spores of coat-defective strains were significantly (P <0.05) more sensitive to PL than the Wt strain, while there was no difference of sensitivity to a UV-C treatment (P > 0.1). PL was inefficient on coat-defective spores when UV-C wavelengths were eliminated. Spores lacking alpha and beta-type small acid-soluble proteins (SASP) were significantly more sensitive (P > 0.05) to PL and 254 nm UV-C exposure than Wt spores. Spores of the *recA* and *uvrB*mutant strains were more sensitive to both treatments than Wt spores.

Significance: Spore coats play a major role in spore resistance to UV applied by PL. The alpha/beta-type SASP and repair of DNA damage during spore outgrowth are some important components of spore resistance to PL, as they also are in the spore resistance to UV. Physical properties of the delivered light (energy of each light pulse, light spectrum,…) can change the effect of a UV-light source on bacterial spore inactivation.

T9-08 Fresh Produce Washing Aid, T-128, Enhances Inactivation of *Salmonella* and *Pseudomonas* Biofilms on Stainless Steel Coupons in Chlorinated Wash Solutions

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Introduction: Bacterial biofilms on food processing equipment can protect pathogens against sanitizers. When chlorine is rapidly depleted by organic materials present in process wash water, inactivation of biofilm pathogens is further challenging.

Purpose: This study was conducted to evaluate the efficacy of chlorinated water (CW) solutions, with or without the washing aid, T-128, on inactivation of *Salmonella* and *Pseudomonas*in biofilms on stainless steel in the presence of increasing organic matter loads.

Methods: Biofilms were formed statically on stainless steel coupons suspended in 2% lettuce extract after inoculation with *Salmonella enterica* serovars Thompson or Newport, or *Pseudomonas fluorescens*. Coupons with biofilms were washed in CW (0, 0.5, 1, 2, 5, 10, or 20 mg/l at pH 6.5, 5.0 and 2.9), with or without T-128, and with increasing loads of organic matter (0, 0.25, 0.5, 0.75, or 1.0 % lettuce extract). Cell populations on coupons were dispersed using intermittent pulsed-ultrasonication-vortexing, and enumerated by colony counts on XLT-4 or *Pseudomonas* agars. Cell responses to fluorescent viability staining after washing treatments were examined using confocal laser-scanning microscopy. Statistical analysis was performed using SAS PROC Mixed procedure.

Results: 0.1% T-128 (without chlorine) reduced *P. fluorescens* biofilm populations by 2.5 logs, but did not reduce *Salmonella* populations. For both bacteria, the sanitizing effect of free chlorine (1.0 - 5.0 mg/l) was enhanced (P < 0.05) by approximately 1.0 - 3.0 logs when combined with T-128. Application of T-128 decreased free chlorine depletion rate caused by increasing organic matter in CW, and significantly (P < 0.05) augmented inactivation of biofilm bacteria. Staining with SYTO 9/propidium iodide corroborated the cultural assay results showing that T-128 can aid in reducing pathogen viability in biofilms.

Significance: T-128 reduced pathogen viability in biofilms when used in CW containing high loads of organic matter. Thus, it can aid in sanitizing surfaces by removing bacterial biofilms during fresh-cut produce processing.

T9-09 Pasteurization or Sterilization of Spices – Food Safety and Quality Considerations

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Introduction: Spices are highly contaminated with pathogenic microorganisms and represent a major hazard for food production and consumption. As not only vegetative microorganisms are present in spices, decontamination normally aims at a reduction of total plate count, which is achieved only if elevated sterilization conditions are applied.

Purpose: It was the purpose of this work to pasteurize and sterilize spices in a steam condensation process and to discuss the sanitation concept optimizing food safety and maximizing quality retention.

Methods: Spices were inoculated with *Enterococcus faecium* NRRL B 2354, a heat-resistant surrogate for *Salmonella*, and with *Geobacillus stearothermophilus*, a heat-resistant surrogate for sporeforming microorganisms. The inoculated products were steam-treated in a controlled condensation process CCP at varying conditions. The inactivation of microorganisms and product quality alterations were assessed.

Results: Product properties with respect to color and volatile oil concentration changed slightly depending on process conditions (time-temperature-moisture-profile), whereas temperature and moisture conditions exhibited a predominant influence. At pasteurization conditions <100°C, the changes in product quality were considerably lower than at sterilization conditions > 100°C. *Enterococcus faecium* was inactivated by more than 5 log units at comparably mild conditions (81-91°C for 5 min). At such conditions sporeformers are hardly harmed but activated. Depending on their heat resistance properties, sporesformers such as *Geobacillus stearothermophilus*were completely inactivated below detection level at elevated process conditions starting from 100°C only.

Significance: These results show that spices may be pasteurized in a validated process at mild and quality-preserving conditions in order to reduce the risk for a *Salmonella* contamination. Although proper pasteurization virtually eliminates the pathogenic risk, total plate counts may still be elevated depending on the initial spore contamination. Hence, successful pasteurization may not be monitored by total plate count analysis. The requirements to validate the pasteurization process on an industrial scale are discussed.

T9-10 Chemical Disinfection of Human Norovirus Surrogates for the Prevention of Human Norovirus Outbreaks

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Developing Scientist Competitor

Introduction: Benzalkonium chloride (BAC), potassium peroxymonosulfate (KPMS), and n-alkyl dimethyl benzyl ammonium chloride (ADBAC) are currently used to decontaminate surfaces, with reported antimicrobial effects against a wide range of pathogenic bacteria and also viruses. However, their effects on human noroviruses have not been studied in detail.

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Purpose: This study determined the effect of 0.32 mg/ml benzalkonium chloride (BAC), 5 mg/ml potassium peroxymonosulfate (KPMS), and a commercial cleanser (ADBAC; recommended concentration of 1:128 dilution) over 1 h at room temperature against human norovirus (hNoV) surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1) *in vitro*.

Methods: FCV-F9 and MNV-1 at titers of ~4 log PFU/ml were treated with equal volumes of three chemicals or water (control) at room temperature for 0, 5, 10, 15, 30, and 60 min. Treatments were neutralized and serial dilutions of treated and untreated viruses were plaque assayed. All treatments were replicated thrice and assayed in duplicate.

Results: Treatment of FCV-F9 with BAC for 5, 10, and 15 min resulted in <1 log PFU/ml reduction, with ~1 log PFU/ml reduction after 30 min, and ~2.8 log PFU/ml after 1 h. FCV-F9 showed no significant titer reduction with ADBAC for 5, 10, 15, or 30 min, with only ~1 log PFU/ml reduction after 1 h. No obvious MNV-1 reduction was obtained after treatment with BAC or ADBAC for 5 or 10 min, but MNV-1 was found to be reduced by ~3 log PFU/ml (based on countable plaques/assay detection limit) after 1 h with BAC, and ~1 log PFU/ml reduction was obtained with ADBAC for 15 min. FCV-F9 and MNV-1 at low titers were reduced to undetectable levels by KPMS for all tested times.

Significance: Our results indicate that KPMS appears to be the most suitable of the three tested chemicals for hNoV surrogate reduction, with broad-spectrum application for clinical and industrial settings.

T9-11 Efficacy of Handwashing Duration and Drying Methods

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Developing Scientist Competitor

Introduction: Handwashing is mandated in the FDA model food code, the guidance used by most US states and territories as the basis for regulating restaurants, grocery stores and institutions such as nursing homes. While the factors that influence handwashing effectiveness have been studied, the data are often based on few replicates and methodological differences or statistical flaws complicate comparisons among studies. This research was undertaken to establish the importance of several key handwashing factors using methods that are robust, sufficiently replicated and statistically valid.

Purpose: Our study quantifies the impact of soap or plain water, duration of the wash (5 vs. 20 s), presence of debris and drying method (air vs. paper towel drying) on the removal of microorganisms from hands during handwashing.

Methods: A food-grade strain of nalidixic acid resistant *Enterobacter aerogenes* was used as a surrogate for transient hand transmitted pathogens like *Salmonella*. Twenty volunteers were used for each experiment. Samples were collected using the glove-juice method where volunteers' hands were massaged for ~1 min inside a nitrile glove containing 20 ml of buffer. Aliquots of the buffer were then plated onto MacConkey agar plus nalidixic acid.

Results: Using soap during handwashing resulted in a greater reduction (~1 log CFU/hand) than using plain water. This difference increased to ~1.5 log CFU/hand when subject hands were contaminated with food debris (5 g of 80% lean ground beef). A food code style 20 s hand wash resulted in ~1.5 log CFU/hand greater reductions than a 5 s wash. Average log reductions for towel drying were ~0.5 log CFU/hand greater than with air-drying; greater person-to-person variability was observed with towel drying.

Significance: This study illustrates the superior effectiveness of handwashing that includes the use of soap and which lasts 20 s. This study also demonstrated that towel drying is more effective than air drying.

T9-12 Eradication of *Salmonella* in a Dry Processing Environment: First Step Moisten, Second Step Decontaminate

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Introduction: Current thought is that for dry processing facilities it is best to keep the environment dry, making the environment uninhabitable for microorganisms. Microorganisms are harder to kill in dry environments, and as a result the options available to decontaminate dry processing areas are very limited. This creates a necessity for exploring other ways to eradicate microorganisms. A new thought is to make the area humid and hospitable to microorganisms prior to decontamination in order to make the microorganisms easier to kill.

Purpose: A study was initiated to validate the efficacy of chlorine dioxide gas (CD) on the inactivation of *Salmonella* Typhimurium at low concentration levels and in short periods of time under dry environments. Traditional exposure for CD to achieve a 6-log sporicidal kill is 720 ppm-hours. As *Salmonella* Typhimurium is non-sporeforming, a much lower exposure level was studied to explore its effect on the bacterium. The long drying time study represents conditions more similar to the actual conditions of a dry processing environment, demonstrating that by keeping the area dry it is increasing the survival capabilities of *Salmonella*. Fumigation using gaseous chlorine dioxide will eradicate all microorganisms to prevent the reoccurrence of persistent contaminations, and the first step for successful decontamination is to raise the humidity.

Methods: Testing was performed inside a 17 ft^3 glove-box isolator with an automated CD generation system for optimal control over all factors being studied. Glass coupons inoculated with *Salmonella* Typhimurium were introduced into the chamber and exposed to 50 – 720 ppm-hr decontamination cycles at 0.3 and 1 mg/l. Drying times before exposure were 2, 24, 72, and 96 hrs. The inoculated and exposed strips were then enumerated and/or enriched along with proper controls to determine the total log reduction.

Results: Under the low-level exposure cycles, CD was able to achieve a 7-log reduction of *Salmonella* Typhimurium at 200 ppm-hrs after a 2-hr drying time. A very important finding was that the longer the drying time was, the more difficult it was to achieve significant log reductions of *Salmonella*. A case study where a 280,000 ft^3 dry protein powder processing facility contaminated with *Salmonella* was decontaminated entirely at one time using chlorine dioxide gas under the findings of the study will also be discussed. Pictures, explanations and data will be provided detailing the entire study.

Significance: Many food processing environments are dry processing environments where there is a necessity for keeping the relative humidity very low (i.e., infant formula production, flour, protein powder, soy powder, etc.). This study shows that humidifying the environment prior to decontamination is an important step towards eliminating microorganisms. The case study displays the real-life ability of chlorine dioxide gas to decontaminate large scale facilities, and how its use along with pre-humidification can eliminate a persistent *Salmonella* contamination.

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T10-01 Comparative Evaluation of an Enrichment Media with a Time-release Selective Agent Tablet for Recovering Nitrite-stressed *Listeria monocytogenes*

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* is a foodborne pathogen that is estimated to cause about 2,500 cases of listeriosis with 500 deaths annually in the United States. The pathogen is often present in a stressed state because of ecological pressure. Isolation from food or environmental sources using traditional enrichment procedures involves manual addition of selective agents during incubation which may cause contamination of media or gradient distribution of selective agents because of inadequate mixing.

Purpose: The purpose of this study was to evaluate a *Listeria*recovery media with a novel proprietary technology developed by 3M™ that incorporates the selective agents into a time-delayed release tablet to overcome these shortcomings.

Methods: Approximately 10 nitrite-stressed (99% injury) cells of *L. monocytogenes* (strain FSL-R2-499) were added to 225 ml each of the following enrichment media: modified *Listeria* recovery broth (mLRB) with core tablets added at time 0 (mC-0) or 6 h (mC-6) after inoculation; mLRB with selective agents manually added at time 0 (mA-0) or 6 hours (mA-6); mLRB with the time-delayed release tablet (mD-6); complete mLRB (mC); UVM; Fraser and Buffered *Listeria* enrichment broth (BLEB) as well as mLRB non-selective and Trypticase soy broth controls. The study was repeated for non-stressed cells as an additional control. Counts of *L. monocytogenes* were determined at 16-26, 40 and 48 h of incubation.

Results: mC-6, mA-6 and mD-6 exhibited similar growth kinetics reaching approximately 4 to 9 log CFU/ml between 16 to 24 h. These levels were slightly higher than mA-0, mC-0, mC, UVM, Fraser and BLEB. mLRB control showed the highest recovery capacity for the pathogen. All the mLRB media showed the highest growth reaching 10 log CFU/ml after 48 h of incubation.

Significance: The time-delayed release tablet would save time, prevent contamination and enable rapid detection of the pathogen at 24 h. Delayed-time release could be indispensable in media for other pathogens requiring addition of selective agents during incubation.

T10-02 Molecular Subtyping of a Large Collection of Historical *Listeria monocytogenes* Strains Using an Improved Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA)

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Introduction: *Listeria monocytogenes* is responsible for rare but severe and often fatal foodborne infections. Accurate and timely molecular subtyping of *L. monocytogenes*isolates is critical to understand the epidemiology of foodborne listeriosis, and to support efforts to minimize listeriosis and outbreak investigations.

Purpose: The purpose of this study was to characterize a large collection of *L. monocytogenes*strains, isolated from Ontario's food chain over the last 15 years, along with Ontario's historical clinical isolates, using MLVA subtyping, to support future preventative efforts.

Methods: Two multiplex PCR reactions were established and optimized under a single condition based on eight specific VNTR loci, which provided high discriminatory power, amplification efficiency and data quality. The fluorescent PCR fragments were separated using an ABI 3730 Genetic Analyzer and analyzed using GeneMapper and BioNumerics software.

Results: Over 2,400 historical *L. monocytogenes* strains of poultry, bovine, swine, produce, environmental and clinical sources were analyzed using the MLVA method. A subset of the strains was also analyzed by standard Pulsed Field Gel Electrophoreses (PFGE) and the results indicated a close alignment in the number of MLVA subtypes and PFGE patterns. Statistical data analysis and strain clustering allowed for identification of distinct clusters, and several predominant and persistent *L. monocytogenes*genotypes in Ontario's food chain, as well as genetic relatedness among various strains. Shared genotypes were identified between food and clinical strains, which may or may not have had any epidemiological link. For instance, the MLVA patterns from the strains of the 2008 Canadian outbreak were also observed among a cluster of 49 strains isolated from chicken, beef, pork, retail and environmental samples between 1998 and 2009.

Significance: The simple, rapid and accurate methodology plus a standardized strain-based DNA fingerprint database will allow for proactive tracking of contamination sources and earlier human cluster detection to minimize foodborne listeriosis.

T10-03 Improvement of Mannitol-yolk-polymyxin B Agar by Supplementing with Trimethoprim for Quantitative Detection of *Bacillus cereus* in Foods

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Developing Scientist Competitor

Introduction: Direct plating on selective media is used as the standard quantitative evaluation method for *Bacillus cereus*in food samples.

Purpose: Mannitol-yolk-polymyxin B agar (MYPA) was modified by supplementation with trimethoprim. The ability of the supplemented medium to select for and recover *Bacillus cereus* from pure cultures and food samples with high background microflora was compared to MYPA.

Methods: For modified MYPA (mMYPA), trimethoprim dissolved in sterilized distilled water with 5 mM HCL was added to cooled MYPA to achieve 32 µg/ml final concentrations. *B. cereus* was experimentally spiked into red pepper powder, soybean paste, vegetable salad, and radish sprout, and then recovered on MYPA and mMYPA for comparison. Twenty-five grams of foods were spiked with 3-5 log CFU/g of *B. cereus*ATCC 14579. Each spiked food sample was suspended in 225 ml of Butterfield's phosphate-buffered water and then homogenized by a stomacher. After stomaching, 0.1 ml of homogenate was serially diluted in 0.85% saline, and then 0.1 ml of each dilution was inoculated onto the two plating media in duplicate. Plates were incubated at 30°C for 24 h and suspected colonies were enumerated. Plates that contained estimated 15-150 suspicious colonies were selected and 5 typical colonies on each plate were sub-cultured. Suspected colonies were finally confirmed with colony PCR. All experiments were repeated three times. Colony counts in food samples were converted to log CFU/g, and analyzed by using ANOVA (Duncan multiple range test).

Results: In all food samples, there was no difference in recoverability ($P > 0.05$) between MYPA (soybean paste, 3.33 ± 0.43; red pepper powder, 3.18 ± 0.20; radish sprouts, 3.33 ± 0.31; vegetable salad, 3.36 ± 0.19) and mMYPA (soybean paste, 3.52 ± 0.47; red pepper powder, 3.34 ± 0.24; radish sprouts, 3.32 ± 0.40; vegetable salad, 3.51 ± 0.23). However, the mMYPA exhibited better selectivity than MYPA, because differentiation and picking of suspected colonies for confirmation of *B. cereus*on MYPA was significantly hindered by dense competing microflora occupying all parts of the plates.

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Significance: Addition of trimethoprim to conventional media could be a useful option to improve selectivity in foods with high background microflora.

T10-04 The *Escherichia coli* Common Pilus: A Diagnostic Target for Point-of-Need LAMP Assays Detecting the Fecal Indicator *E. coli*

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Developing Scientist Competitor

Introduction: Pathogens derived from fecal contamination of food and water pose significant public health concerns, yet assays to identify fecal indicator bacteria (FIB) at the point-of-need are typically limited to slow culture based techniques. Molecular assays can accelerate the identification of FIB, but currently these assays are not conducive to point-of-need detection because of their expense, need for specialized equipment, and need for highly trained personnel. Thus, new molecular detection technologies are needed for FIB identification.

Purpose: Here, we describe a field-capable molecular detection platform to rapidly identify the FIB *Escherichia coli* in irrigation water and seawater. This platform relies on an advanced nucleic acid amplification strategy, loop-mediated isothermal amplification (LAMP), and field-ready instrumentation to monitor the progression of LAMP reactions.

Methods: A LAMP assay was developed targeting a portion of *ecpA*, the major pilus subunit of the *E. coli* common pilus (ECP), and evaluated with genomic DNA from 15 isolates of pathogenic *E. coli* (representing six common Shiga toxin-producing *E. coli* serotypes, as well as O104:H4, and O157:H7). Efficacy of the LAMP reaction was also tested by detecting naturally present *E. coli*filtered from seawater (obtained from oyster beds) and spiked irrigation water.

Results: LAMP reactions targeting *ecpA* reliably detected 1 pg of genomic DNA from all isolates tested, the equivalent of approximately 2.5×10^2 bacteria, in less than 25 minutes. Detection times were dependent on the concentration of template DNA (100 ng of DNA was detectable in less than 13 minutes), thus allowing for semi-quantitative estimates of bacterial contamination. Further, LAMP detection of *E. coli*was successful in filtrates of seawater and spiked irrigation water.

Significance: These results demonstrate the feasibility of utilizing the ECP of *E. coli* as a diagnostic target, and the studies presented here provide the foundation for the development of rapid, point-of-need, LAMP assays to evaluate fecal contamination of food and water.

T10-05 Evaluation of a Novel Microbial Source Tracking Method for Identification of Fecal Contamination in the Fresh Produce Production Environment

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Developing Scientist Competitor

Introduction: Traditional microbiological indicators of fecal contamination cannot discriminate between contamination sources (i.e., humans, ruminants, reptiles, etc.). Microbial Source Tracking (MST) methods, commonly used in environmental microbiology, can be used to track fecal contamination by source. Those MST methods based on *Bacteroidales*are particularly promising.

Purpose: To investigate the utility of a 16S rDNA *Bacteroidales*subtying method for identification of fecal contamination in the fresh produce production environment.

Methods: Initial efforts focused on optimizing a method to detect total *Bacteroidales* in environmental (lake water) samples using pre-analytical sample processing, DNA extraction and quantitative real-time PCR (qPCR). A combination of filtration and centrifugation was chosen for sample processing, followed by DNA extraction and qPCR amplification using the previously reported AllBac primer/probe combination. A homologous internal amplification control was also developed for inclusion in qPCR to identify those samples having residual matrix-associated inhibitors. The optimized methods were used to detect *Bacteroidales* contamination in 70 buffer rinsates of fresh produce (cantaloupe, tomatoes, melons), harvester hands, source, and irrigation waters originating from Northern Mexico. The same samples were also screened for the presence of generic *E. coli*.

Results: The optimized method was able to detect 2 mg feces per 100 ml water sample. Of the 70 samples obtained from Northern Mexico, 59/70 (84%) were successfully screened for *Bacteroidales*, while 11/70 (16%) were uninterpretable because of excessive levels of PCR inhibitors. Overall, 28/59 (48%) samples showed evidence of *Bacteroidales* contamination. When analyzed across sample types, positivity was 59%, 25%, and 50% for the produce rinsates, waters, and hand rinses, respectively. Concordance between *Bacteroidales* and generic *E. coli* was 46%.

Significance: These results demonstrate that *Bacteroidales* 16S rDNA can be detected in environmental samples collected from fresh produce production. Additional studies are underway to determine the usefulness of species-specific (e.g., human and ruminant) *Bacteroidales* assays to delineate fecal contamination source.

T10-06 Colorimetric Paper-based Detection of *Salmonella* spp. and *Escherichia coli* from Artificially Contaminated Irrigation River Water

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Developing Scientist Competitor

Introduction: Irrigation water serves as a major source of contamination of fresh produce with bacterial foodborne pathogens including *Salmonella* spp. or *Escherichia coli* O157:H7, and timely testing of irrigation water may prevent this contamination. These methods should be sensitive, inexpensive, and have field applicability. Paper-based analytical devices (μPAD) are inexpensive and may provide an effective way of rapidly testing for bacterial pathogens in irrigation water.

Purpose: The purpose of this study was to develop and evaluate an integrated colorimetric paper-based detection assay for rapid, sensitive and simultaneous detection of *Salmonella* spp. and the fecal indicator bacteria *E. coli*in irrigation water.

Methods: Large volumes (10 l) of irrigation water were spiked with three-strain cocktails of either *Salmonella* spp. or *E. coli* at concentrations of 0.1 CFU/ml to 100 CFU/ml. Inoculated samples were concentrated using Moore Swabs (MS) or disposable inline filters (DIF) prior to enrichment in buffered peptone containing selective supplements (25 ml samples of pre-concentrated samples were also enriched). Enrichment was performed at 42°C and colorimetric testing was performed at 8, 12 and 18 hours. μPADs employed the following enzyme/substrate pairs β-galactosidase/chlorphenyl red β-galactopyranoside (CPRG) and esterase/5-bromo-6-chloro-3 indolylcaprylate for colorimetric detection of *E.*

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coli and *Salmonella*spp., respectively.

Results: Detection of *Salmonella* spp. and *E. coli* was possible at concentrations as low as 0.1 CFU/ml after 8 hours enrichment. Significantly ($P < 0.001$) more spiked samples were detected following concentration using MS or DIF compared to non-concentrated samples. Within 8 hours, *E. coli* could be detected at 0.1 CFU/ml only after concentration, while for *Salmonella* this was true for all concentrations except 100 CFU/ml.

Significance: The results of this study indicate that μPADs can be used to rapidly and inexpensively detect *Salmonella* spp. and *E. coli* in irrigation water, and can be potentially employed in a field setting for rapid and sensitive detection of bacterial pathogens.

T10-07 Development of Latex Agglutination Tests for Non-O157 Shiga Toxin-producing *Escherichia coli* O26, O45, O103, O111, O121 and O145

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Introduction: Shiga toxin-producing Escherichia coli (STEC) strains are important foodborne pathogens responsible for numerous outbreaks. The top six non-O157 STECs (O26, O45, O103, O111, O121 and O145) are responsible for 70% of non-O157 human illness in the United States.

Purpose: Simple and rapid methods to detect and identify these pathogens are needed to help confirm these serogroups. Latex agglutination assays were developed for detection of the top six non-O157 STECs.

Methods: Immunoglobulins (IgG) were affinity purified from rabbit antisera and covalently immobilized onto polystyrene latex particles. The optimum conditions for the agglutination assay consisted of utilizing 20 μl of latex-IgG reagent containing 2.0–2.8 mg IgG in a 0.5% latex suspension.

Results: Agglutination was observed instantly after mixing the colonies with the latex-IgG indicating positive reactions for the target strains. This method detected and helped confirm the target serogroups. Over 100 target and non-target strains were tested in more than 3,000 test replicates. The anti-O103 and anti-O145 latex reagents showed cross-reactions with O26 strains while anti-O26 cross-reacted with serogroup O103. The latex-IgG reagents are stable for at least one year.

Significance: The latex-IgG reagents can be used for identification of presumptive positive non-O157 STEC colonies. The method of preparation of the latex reagents can also be utilized to prepare these types of reagents for other STEC serogroups and other pathogens to ensure safe foods to consumers.

T10-08 Continuous Aerosol Sampling of Interstitial Headspace for Post-process Contaminants in Dry Foods

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Introduction: Detection of post-process biological and chemical contaminants in finished food products are challenging due to the limitations of detecting contaminants that are not typically randomly distributed. A novel device for aerosol-based sampling of the interstitial headspace of low-moisture foods has been developed which can continuously sample food for contaminants.

Purpose: The goal of this study was to compare the detection of an aerosol-based sampling device to traditional grab sampling for the detection of milk allergen, GloGerm or food surface adhered *Enterococcus faecium* NRRL B-2354.

Methods: In the first pilot plant study, five grab samples (25 g) each were secured at random from totes of puffed rice cereal (50 lbs), previously spiked in one location with 0.1 g of GloGerm or 0.5 g of dry milk powder. Following sampling, cereal was gravity fed through the device that samples the interstitial headspace (i.e., aerosol) and suspends the contaminant in a 10 ml cartridge containing sterile buffer. In the second pilot plant study 25 lbs of puffed rice cereal were spiked in one location with 30 g of food surface adhered *Enterococcus faecium* NRRL B-2354 (10^7 log CFU/g) or 1.0 g of dry milk powder. The inoculated cereal was gravity fed through the sampling device, packaged into 50 g cereal bags and subsequently collected (30 total, every fourth bag). *Enterococcus faecium*, milk allergen and GloGerm in the puffed rice cereal and liquid cartridges were analyzed using TSA/KF media, ELISA and fluorescence spectrophotometry.

Results: This novel sampling device successfully detected the contaminating milk, GloGerm and *E. faecium* in both studies while the grab sampling approach detected 1 positive out of 15 samples for dry milk powder, and 0 positive in 15 samples for GloGerm. In study 2 the grab samples contained the contaminant in 48.3% and 33.3% of the finished food product for *E. faecium* and milk allergen, respectively. The packaging equipment configuration randomized the contaminant distribution more in study 2 relative to study 1.

Significance: This novel aerosol-sampling device has the potential to continuously detect post-process biological and chemical contaminants at a higher incidence relative to traditional grab sampling.

T10-09 Development of New Methods for Potential Detection of *Blastocystis* sp. in Fresh Produce

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Introduction: The zoonotic parasite, *Blastocystis* sp., has been regularly detected in surface water and on leafy vegetables. Surveillance studies revealed that *Blastocystis* infection is very common among food handlers worldwide. Prevalence of*Blastocystis* in food industry personnel ranged from 4.87% in Turkey, to 35.5% in Egypt, 38.7% in Venezuela, 41.5% in Thailand, and 41.7% in Mexico. In the US, *Blastocystis* is one of the most frequently found parasites in humans and is prevalent in livestock.

Purpose: To develop immunofluorescence microscopy and molecular techniques to facilitate detection and identification of *Blastocystis sp.* in produce.

Methods: Conserved regions of published nucleotide SSU rDNA sequences of *Blastocystis* (available from GenBank) were used to develop a set of primers for PCR. A new immunoflourescence microscopic test for *Blastocystis sp.* (Boulder Diagnostics) was evaluated for sensitivity and specificity by conducting *Blastocystis* detection in biologic samples containing multiple zoonotic protozoan pathogens such as *Giardia duodenalis*, *Cryptosporidium* spp., and *Enterocytozoon bieneusi*.

Results: A specific pair of PCR primers was successfully developed for the identification and subtyping of *Blastocystis*. The specificity of these primers was confirmed by the successful amplification of DNA from all *Blastocystis* specimens and their inability to amplify DNA from other protist parasites present in the sample. For the first time the successful immunofluorescence detection of *Blastocystis* in complex poly-protist samples was completed.

Significance: Development and application of superior detection and identification procedures for *Blastocystis* are needed to evaluate the true prevalence as well as routes of parasite transmission and contamination of fresh produce. The methods described herein are potentially applicable to screening of fresh produce.

Technical

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T10-10 Rapid Detection of *Salmonella* spp. in Foods and Environmental Samples Using Isothermal Nucleic Acid Amplification

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Introduction: A rapid *Salmonella*spp. detection method (ANSR™) has been developed based on the nicking enzyme amplification reaction (NEAR™) technology. The test chemistry employs isothermal nucleic acid amplification and real-time detection using fluorescent molecular beacon probes.

Purpose: The purpose of the study was to measure inclusivity and exclusivity characteristics of the method and to assess method performance in testing of various types of environmental samples.

Methods: Inclusivity and exclusivity was determined in testing of target and non-target bacteria in pure culture utilizing enrichment protocols specified for the method. Environmental sample testing consisted of sponge or swab samples taken from five types of environmental surfaces (stainless steel, plastic, ceramic tile, sealed concrete, and rubber) inoculated with *Salmonella*spp. and in some cases co-inoculated with a cocktail of competitor bacteria. Performance of the Isothermal Nucleic Acid Amplification method was compared to that of the U.S. FDA reference culture procedure.

Results: Results of inclusivity testing showed that the method is specific for *Salmonella* spp. and inclusive for serovars of both *S. enterica* and *S. bongori*. Of a total of 145 environmental samples tested, there were 69 and 71 samples positive by the Isothermal Nucleic Acid Amplification method after 16 and 24 hours of enrichment, respectively, compared with 68 samples positive by the reference method. For one environmental surface type, stainless steel, there was a significant difference in method performance, in favor of the Isothermal Nucleic Acid Amplificationmethod, as determined by chi-square analysis. Performance of the Isothermal Nucleic Acid Amplification and reference methods was not statistically different for the other four sample types.

Significance: The Isothermal Nucleic Acid Amplification method represents an advance in molecular testing for *Salmonella* spp. in foods and environmental samples. Single-step enrichment protocols are used, equipment and labor requirements are minimal, and the Isothermal Nucleic Acid Amplification assay can be completed in 30 minutes following enrichment.

T10-11 Homogenous Detection of Fumonisin B1 with Molecule Beacon Based on Fluorescence Resonance Energy Transfer between Upconversion Nanoparticles and Gold Nanoparticles

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Introduction: The Fumonisin B_1 is a nephrotoxin in all species tested; a carcinogen and a reproductive toxicant in rodents, likely in humans. They are most frequently found in corn, corn-based foods and feeds, and other grains such as sorghum and rice. Rapid and effective measurement of Fumonisin B_1 is of great importance for food safety.

Purpose: We presented a new aptamer biosensor for Fumonisin B1, which was based on fluorescence resonance energy transfer (FRET) between upconversion fluorescent nanoparticles (UCNPs) and gold nanoparticles (Au NPs).

Methods: The quencher (Au NPs) were attached to 5 end of the molecular beacons (MB) and the donor (UCNPs) attaching to 3 end of the MB. In the absence of target DNA (complementary DNA to FB1 aptamer), the energy donor and acceptor were taken into close proximity, leading to the quenching of fluorescence of UCNPs. Due to the combination between FB1 and FB1-specific aptamer, and caused the some complementary DNA dissociating from magnetic nanoparticles (MNPs). In the presence of the complementary DNA, the MB underwent spontaneous conformational change and caused the UCNPs and Au NPs to detach from each other, resulting in the restoration of UCNPs fluorescence.

Results: The fluorescence of UCNPs was restored in a FB_1 concentration-dependent manner, which built the foundation of FB1 quantification. The biosensor provided a linear range from 0.01 to 100 ng/ml for FB1 with a detection limit of 0.01 ng/ml in an aqueous buffer. As a practical application, the biosensor was used to monitor FB_1 level in naturally contaminated maize samples with the results consistent with that of a classic ELISA method, indicating that the UCNPs-FRET biosensor was competent for directly sensing FB_1in foodstuffs samples without optical interference, which benefited from the near infrared (NIR) excitation nature of UCNPs.

Significance: This work opened the opportunity to develop biosensors for mycotoxins using the developed FRET system.

T10-12 Easy, Rapid, and Cost-effective Real-Time PCR Detection of Norovirus GI and GII with a Single Tube Lyophilized One-step Reverse Transcription PCR Mix

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Introduction: Infection with norovirus is a major cause of food- and water-borne gastroenteritis worldwide. According to CDC, in the United States alone it is estimated that 50% of foodborne disease outbreaks are caused by noroviruses. The gold standard for rapid detection of norovirus is reverse transcription PCR, but current PCR based methods are time consuming, labor intensive, and generally difficult to perform.

Purpose: The goal of this study was to develop and evaluate a single tube temperature-stabilized lyophilized one-step reverse transcription mix that includes primers and probes for detection and genogrouping of noroviruses.

Methods: We designed a multiplex, real-time, one-step reverse transcription PCR targeting norovirus GI and GII, the genogroups most commonly associated with human illness. The single-tube triplex assay includes an RNA internal amplification control to evaluate samples for inhibitory compounds that might produce false-negative results. For ease of use, all of the assay components are lyophilized into a ready-to-run, "Sample-Ready™" reaction mix. One need only add the RNA sample in water to rehydrate the mix, and insert the tube into a real-time PCR instrument. Benefits of this assay include storage at 4°C, no manual mixing or micropipetting of enzymes, buffers, primers, or probes, and no requirement for pre-incubation or post-PCR steps. Test results are reported in under 1 hour.

Results: In testing with synthetic RNA controls, the assay demonstrates sensitivity down to the single genomic copy level. The assay was evaluated for specificity against a panel of norovirus-containing samples isolated from numerous outbreaks and produced excellent correlation to detection and genogrouping results from previous studies with these isolates.

Significance: This novel assay provides public health agencies and the food industry with a much needed more rapid and easy to run method for the detection and genogrouping of noroviruses associated with human illness. The multiplex detection and lyophilized formulation also make the assay cost-effective to run in terms of test and labor costs required for analysis.

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Poster Abstracts

P1-01 Preliminary Evaluation of VIDAS® UP *Listeria* (LPT) Assay for the Detection of *Listeria* in Select Food and Environmental Surface Samples

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Introduction: The detection of *Listeria* in food and environmental surface samples using single enrichment approach without compromising the sensitivity could help to enforce food safety objectives. Based on a specific phage capture technology, VIDAS®UP *Listeria* (LPT) assay is a new generation of automated qualitative test with the capability to detect *Listeria*within 26-30 h in food and 22-30 h in environmental surface samples.

Purpose: To perform a preliminary evaluation of LPT assay to detect low levels of *Listeria*(0-5 CFU/ sample) in artificially contaminated food and environmental surface samples with comparison to reference method.

Methods: Twenty-five gram samples (ham, turkey sausage, salmon and spinach) of each food matrix were diluted (1:10) in proprietary LPT broth and incubated at 30 °C for 26-30 h. Two types of environmental samples including sponges from ceramic tiles, and cotton swabs from stainless steel, plastic and rubber surfaces were also enriched in LPT broth at 30 °C for 22-30 h. All enriched samples (n=20) were analyzed using LPT assay and simultaneously confirmed on selective media. The reference method (FDA-BAM or USDA-MLG) was performed in parallel for all samples.

Results: The results of LPT assay and reference method for any tested foods were not found statistically significantly different. Matrix-wise results (positives for LPT/reference) were 19/19 for ham; 5/5 for sausage; 11/9 for salmon; and 5/5 for spinach. For environmental samples phage-based assay either performed statistically better or equivalent to the reference method. The results for environmental samples were 14/9 for sponges from ceramic tiles, and 6/5 for swabs from rubber, 15/14 from plastic and 15/4 from stainless steel surface.

Significance: The evaluations of LPT assay provided satisfactory results for the detection of *Listeria* in artificially contaminated food and environmental surface samples and uninoculated controls. This automated assay could provide an easy to use solution for the detection of low levels of *Listeria* in a sensitive and rapid manner.

P1-02 Performance Tested Method℠ Evaluation of the Roka *Salmonella* Detection System for Food and Environmental Surfaces on the Atlas™ System

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Introduction: *Salmonella*has been implicated as a major cause of human foodborne illness worldwide. There is an increased demand to apply effective detection methods that are rapid, accurate and easy to use.

Purpose: To evaluate the Roka *Salmonella* Detection Assay system for the detection of *Salmonella enterica spp*. in food and environmental surfaces in an AOAC Research Institute *Performance Tested Method* ℠ study utilizing Roka's high throughput, fully automated instrument – Atlas™ System.

Methods: The method included a single 12 or 16 hours enrichment for perishables or 24 hours enrichment for non-perishables and environmental surfaces. After sample transfer to the instrument collection tube, lysis of bacteria, template specific sample extraction, amplification and probe detection were all performed on the instrument. A total of 12 foods, including two 375-g composites, and 3 environmental surfaces were compared to a cultural reference method in an internal study; 4 foods and 1 environmental surface in an external study. Selectivity was evaluated by testing 100 target microorganisms and 30 non-target microorganisms.

Results: The test method provided a positive result for 100% of 100 target microorganisms, and a negative result for 30 non-target microorganisms. No significant differences were observed between the test method and the cultural reference method for raw ground beef, raw ground chicken, deli-cooked turkey, raw cod, dried milk, string cheese, milk chocolate, cocoa powder, cookie dough, nacho cheese seasoning, sealed concrete and plastic. Dried egg in the internal study and peanut butter in the external study had a higher number of confirmed test portions for *Salmonella* compared to the cultural reference method; stainless steel had a lower number in the external study only. The test method provided the final result in 18-30 hours utilizing a fully automated instrument compared to at least 3 days for cultural methods.

Significance: *Salmonella enterica* spp. can be detected at low levels of contamination in as few as 12 hours of enrichment.

P1-03 Performance Tested Method℠ Evaluation of a Novel *Listeria* Detection Assay for Food and Environmental Surfaces

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Introduction: Listeriahas been implicated as a major cause of human foodborne illness worldwide. There is an increased demand to apply effective detection methods that are rapid, accurate and easy to use.

Purpose: To evaluate the Roka Listeria Detection Assay system for the detection of Listeria spp. in food and environmental surfaces in an AOAC Research Institute Performance Testing Methods (PTM) study utilizing Roka's high throughput, fully automated walk-away instrument – Atlas™ System.

Methods: The method includes 24-28 hours enrichment at 35°C for foods and environmental surfaces in Half-Fraser media. Enriched sample is transferred to a collection tube containing lysis reagent and loaded onto the Atlas™instrument where template-specific sample extraction, TMA amplification, probe detection and analysis of the results are all performed. A total of 9 foods and 3 environmental surface matrices are compared to a cultural reference method in an internal study, as well as, 2 foods and 1 environmental surface in an external study. Selectivity was evaluated by testing 50 target microorganisms and 30 non-target microorganisms.

Results: The test method provided a positive result for 100% of 50 target microorganisms, and a negative result for all 30 non-target microorganisms commonly found in food and grown to a titer > 1+E08 CFU/ml. No significant differences were observed between the Roka Listeria Detection Assay and the cultural reference methods for cured ham, chicken salad, deli chicken, brie cheese, pasteurized whole milk, cold smoked salmon, romaine lettuce, hot dog inoculated with L. grayi, stainless steel and sealed concrete. Significant differences were observed with Roka's method demonstrating higher sensitivity to detect positives in vanilla ice cream, PVC plastic and hot dog inoculated with *L. innocua*.

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Significance: Roka's method provides final negative or presumptive positive results in 24-28 hours compared to at least 3 days for culture methods. *Listeria spp*. can be detected at low levels of contamination and combined with the Atlas™System offers a rapid, specific, and user-friendly test method to monitor and limit contamination issues.

P1-04 A New Phage-based Immunoassay for Detection of *Listeria* in Food and Environmental Samples

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Introduction: A new immunoassay, the VIDAS®UP *Listeria* (LPT) for detection of *Listeria*species in food and environmental surfaces was developed. This assay incorporates phage proteins to increase the sensitivity and specificity compared to traditional immunoassays.

Purpose: The study was undertaken to validate the LPT method as part of the AOAC RI approval process.

Methods: In the new method, food samples are enriched in LPT broth (26-30 h for food, 22-30 h for swabs) then 0.5 ml is transferred to the VIDAS strip, heat treated for 5 minutes and cooled before performing the assay (62 min). Five meat products (hot dogs, pepperoni, deli ham, chicken nuggets and liver pate) and 4 surfaces (plastic, stainless, ceramic and concrete) were included in the study. For each matrix, twenty samples inoculated at a low level (target 0.2-2 CFU) and 5 uninoculated samples were tested using the LPT assay and the USDA reference method.

Results: The LPT method gave comparable results to the USDA method using an unpaired Chi-square test at the 5% level. Results were available within 28 h for food samples and 24 h for environmental swabs.

Significance: The LPT test is simple to perform and allows next-day detection of *Listeria* species in food products or manufacturing environments.

P1-05 What's Up with VIDAS UP?

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Introduction: After *Campylobacter*, *Salmonella*is the second most common cause of human foodborne gastroenteritis in the developed world, with poultry being a significant vehicle. The ISO 6572:2002 cultural method, commonly applied to poultry, is labor, materials and lead time intensive. The VIDAS UP assay is one of many recently developed alternative, rapid methods.

Purpose: To compare the ISO method to the VU assay for analysis of rinses of raw poultry, with respect to agreement (equivalence?), as well as cost and time benefits.

Methods: The ISO method was compared to the AOAC-approved VU protocol. The latter was also modified by inclusion of a 2-4 or 12 h pre-enrichment as well as, post-assay, selective enrichment using RVS medium or immunoconcentration (VIDAS ICS protocol), prior to plating to SMID agar. All protocols were tested using 90 raw poultry samples. All data points were analyzed statistically. A time and motion was performed for each of the assays using a stopwatch and 30 samples.

Results: There was disagreement between the two analytical methods when used without modification, due to overgrowth by the background flora upon plating for confirmation. Reliable detection of *Salmonella* from raw poultry required a minimum of a 2 h pre-incubation prior to addition of the VU selective supplement. Following the UP protocol, selective enrichment using RVS medium or the VIDAS ICS was required to reduce breakthrough organisms such as *E. coli*, *Citrobacter* and*Klebsiella* spp. Likely due to low numbers and random distribution, 100% agreement between methods is unlikely. The VU method was more efficient with respect to labour (75% reduction), materials (25% cost reduction), and lead time (40-67% reduction).

Significance: The modified VU assay provides efficiencies in laboratory operation and timely results when monitoring CCPs in poultry processing.

P1-06 Detection of Five Shiga Toxin-producing *Escherichia coli* Genes with Multiplex PCR

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Introduction: *Escherichia coli* serogroup O157 remains most commonly associated with foodborne outbreaks, but epidemiological studies suggest that non-O157 Shiga toxin-producing *E. coli* (STEC) causes 20-50% of STEC infections accounting for approximately 169,000 illnesses annually in the U.S. The ten most clinically relevant STECs belong to serogroups O26, O103, O111, O145, O157, O91, O113, O128, O45 and O121. Emerging strains such as the O104:H4 identified with the 2011 German outbreak could become more prevalent in the future.

Purpose: The purpose of this study is to develop a conventional multiplex PCR assay for the specific detection of STEC.

Methods: The multiplex PCR for STEC serotypes, O26, O45, O91, O103, O104, O111, O113, O121, O128, O145 and O157:H7 targets 5 different genes, *stx1, stx2, eaeA, ehxA and uidA. To validate this PCR, 135 STECs, including one O157:H7 and three O157, were used for inclusivity (sensitivity) testing and 30 non-STECs were used for exclusivity (specificity) testing.*

Results: In 1.5 hours, from PCR analysis to results, all of the STEC DNA showed the presence of 1 to 5 amplification products, while the non-STEC DNA did not react to this multiplex PCR assay. In addition to being able to confirm O157:H7 isolates, it can identify non-O157:H7 STEC isolates that the current *Bacteriological Analytical Manual* O157:H7 multiplex PCR assay cannot detect. The *uid*A+93 SNP was retained in the assay to differentiate between O157:H7 and non-O157:H7 strains.

Significance: The rapid detection of STEC genes with the multiplex PCR assay will enable the rapid confirmation of virulent non-O157 STEC and O157:H7 food isolates, thus protecting the public health by enabling the identification and removal of these adulterated foods from the nation's food supply.

P1-07 Comparison of Phenotypic and Genotypic Subtyping Methods for Differentiating *Salmonella* Enteritidis Isolates Obtained from Food and Human Source

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Developing Scientist Competitor

Introduction: *Salmonella* typing technologies are essential for bacterial source tracking and to determine the distribution of pathogens isolated from ill people. Traditional typing methods based on their phenotypic traits, such as biotyping, antibiotic susceptibility profiles, serotyping and phage typing provide insufficient information for epidemiological purposes. Molecular subtyping methods have revolutionized the fingerprinting of microbial strains, but most of them have not been internationally standardized.

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Purpose: In this study, we evaluated the abilities of two phenotypic subtyping methods (phage typing and antimicrobial susceptibility) and three genotypic subtyping methods (PFGE, rep-PCR and MLST) to distinguish among a collection of*S. enterica* Enteritidis (*S.* Enteritidis) isolates that were collected from food and human sources.

Methods: We determined subtypes of *Salmonella* Enteritidis (*S.* Enteritidis) isolated from food products (*n* = 10) and human clinical samples (*n* = 10) between 2009 and 2010 in Seoul using five subtyping methods and evaluated the abilities of these subtyping methods. These methods included phage typing, antimicrobial susceptibility, PFGE, Rep-PCR and MLST methods. We compared the abilities of three different subtyping methods to distinguish *S. enterica* Enteritidis (S. Enteritidis) by calculation of Simpson's diversity index.

Results: Among the 20 isolates tested, there were six antimicrobial susceptibility patterns, three different phage types, four different PFGE profiles, seven Rep-PCR patterns and one MLST type. Food isolates were considerably more susceptible to antibiotics than human isolates. We were best able to discriminate among *S.* Enteritidis isolates using Rep-PCR, and obtained the highest Simpson's diversity index of 0.82, while other methods produced indices that were less than 0.71. PFGE pattern appeared to be more related to antimicrobial resistance profiles and phage types of *S.*Enteritidis isolates than rep-PCR. MLST revealed identical alleles in all isolates at all seven loci examined, indicating no resolution.

Significance: The results of this study suggest that Rep-PCR provided the best discriminatory power for phenotypically similar *S.* Enteritidis isolates of food and human origins, while the discriminatory ability of MLST may be problematic due to the high sequence conservation of the targeted genes.

P1-08 Robustness of Loop-mediated Isothermal Amplification Assays for *Salmonella* Detection

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Developing Scientist Competitor

Introduction: *Salmonella* is a leading cause of foodborne illnesses and deaths in the United States and worldwide. Molecular-based methods such as PCR and more recently, loop-mediated isothermal amplification, have gained wide applications in *Salmonella*detection, due to their rapidity, specificity and sensitivity. However, there is a paucity of data regarding the robustness of these assays.

Purpose: This study aimed at evaluating the robustness of two recently developed loop-mediated isothermal amplification (LAMP) assays for *Salmonella* detection, using PCR as the comparison method.

Methods: Performances of the LAMP and PCR assays were examined under various abusive assay preparation and running temperatures, pH and in the presence of potential inhibitors in food applications (media used for enrichment and dilution, plant polysaccharide, humic acid) and actual food rinses (chicken and ground beef).

Results: The LAMP assays achieved robust detection of *Salmonella*cells under abusive assay preparation (25 and 37°C with holding up to 30 min) and running temperatures (60-68°C). By using a hot start DNA polymerase, PCR obtained comparable results under these temperature ranges. However, PCR performed markedly poorer under abusive pH values. In the presence of inhibitors, LAMP assays also demonstrated greater tolerance than PCR. When chicken and ground beef rinses were added at 20% into the reaction mix, PCR amplifications were completely inhibited, but LAMP reactions were not.

Significance: LAMP assays were demonstrated to be a robust alternative to PCR in detecting *Salmonella* under abusive reaction conditions and with inhibitors. Therefore LAMP could be adopted for routine *Salmonella* testing in food samples with rapidity and robustness.

P1-09 Monitoring *Campylobacter* Population on Poultry by Magnetostrictive Biosensor

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Introduction: The incidence of *Campylobacter* on chicken carcasses is high; around 60% of rinsed carcasses are positive. This problem creates the need for rapid detection methods for the poultry industry to monitor *Campylobacter* on chicken and chicken products. The magnetostrictive particles (MSP) biosensor which is operated wirelessly can easily be the candidate for this purpose.

Purpose: The purpose of this research was to develop a sensitive and cost-effective MSP biosensor for on-site quantitatively monitoring *Campylobacter* to improve the efficacy of HAACP plans.

Methods: The sensors were coated with silica through the treatment of tetraethoxyslane and gold coated by direct sputtering. Anti-*Campylobacter jejuni* antibodies were immobilized on both coated sensors and the immobilization efficiency was measured by ELISA. The performance of MSP biosensors were tested in the inoculated phosphate buffer and poultry processed water.

Results: MSP sensors with the size of 1.0 mm × 0.2 mm × 0.025 mm were fabricated and coated with one layer of 100 nm gold or 30 nm silica, respectively. Both sensors were immobilized with anti-*Campylobacter* rabbit IgG. Based on the ELISA test, the silica coated sensors have higher antibody immobilization efficiency (O.D. 405 nm=1.4240) than the gold coated sensors (O.D. 405 nm=1.0114). The silica coated biosensor has higher binding efficiency to *Campylobacter jejuni* than those coated with gold. The detection limits for both biosensors were around 10^2-10^3 CFU/ml.

Significance: The results have shown that the MSP biosensor has low detection limits for *Campylobacter jejuni.* There is a high potential to develop a low-cost, high sensitivity handheld device of MSP biosensor for application in the poultry processing plants to monitor the *Campylobacter* population increasing the control efficacy.

P1-10 Modification of Charcoal-cefoperazone-deoxycholate Agar by Supplementation with a High Concentration of Polymyxin B for Detecting *Campylobacter jejuni* and *Campylobacter coli* in Chicken Carcass Rinse

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Developing Scientist Competitor

Introduction: Although culture with selective media is the standard method for detecting *Campylobacter,*it is difficult to isolate the bacteria from raw chicken with numerous background microflora. We improved modified charcoal cefoperazone deoxycholate agar (mCCDA) by adding polymyxin B to enhance selectivity of the media.

Purpose: The aim of this study was to evaluate the effect of high concentration of polymyxin B on the detection ability and selectivity of mCCDA to isolate *C. jejuni* and *C. coli*from chicken carcass rinse.

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Methods: Modified mCCDA (P-mCCDA) was made by adding 100,000 IU of polymyxin B to 1 liter of mCCDA. Chicken carcasses were rinsed with 400 ml of buffered peptone water. To ensure even distribution, the carcass rinse was mixed thoroughly by gently shaking the sample for 1 min. A 25 ml test portion from the 400 ml rinse was enriched with 25 ml of 2× blood-free Bolton enrichment broth in a 50-ml screw-cap conical tube. Less than 0.5 cm of headspace was left in the tubes and the lids were tightly capped. Each sample (50 ml) was enriched at 42°C for 48 h. A loopful of the enrichment broth was streaked onto mCCDA, Campy-Cefex agar, and P-mCCDA followed by incubation under microaerobic condition at 42°C for 48 h. Suspected colonies (maximum-10) were removed and sub-cultured onto 5% horse blood agar. Presumptive *C. jejuni* and *C. coli* colonies were confirmed with colony PCR. We compared the number of plates of *C. jejuni/ C. coli* and competing organisms in terms of isolation rate and selectivity, respectively.

Results: The number of P-mCCDA (70 out of 80) plates positive for both *C. jejuni* and *C. coli* was significantly higher ($P < 0.05$) than those of positive mCCDA (51 out of 80) and Campy-Cefex (36 out of 80) plates. Furthermore, fewer ($P < 0.05$) P-mCCDA plates (31 out of 80) were contaminated than other media plates (mCCDA, 34 out of 80; Campy-Cefex, 79 out of 80), indicating the superior selectivity of P-mCCDA.

Significance: Addition of high concentration of polymyxin B to conventional media could be a useful option to improve selectivity and isolation rate in chicken carcass rinse.

P1-11 Comparison of an Automated Most Probable Number (MPN) Technique to a Manual Method for the Enumeration of Total Aerobic Counts in Food Contact Surface Samples

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Introduction: The enumeration of total aerobic counts in food contact surface samples is critical when assessing the sanitary state and bacterial load in the food production environment. Traditional microbial enumeration techniques consist of time-consuming and labor-intensive steps including serial dilution preparation, plating and colony counting. The TEMPO®TVC method is an automated system for the enumeration of total aerobic mesophilic flora that eliminates these objectionable steps. The method uses an enumeration card containing 48 wells across three different dilutions for the automatic determination of MPN.

Purpose: To compare an automated MPN based system to a manual method for the enumeration of total aerobic counts in food contact surface samples from a commercial chicken production facility in the USA.

Methods: Fifty food contact surface swab samples were collected randomly from a poultry production facility and were processed to enumerate the total aerobic counts using the TEMPO®TVC system and Petrifilm™ Aerobic Count Plates. The log values of counts (CFU/ml) enumerated by each method were analyzed statistically for the calculation of overall bias and correlation coefficient between the methods.

Results: A 95% confidence interval of the bias was calculated for the log values (CFU/ml) of total aerobic counts enumerated by each method. The calculated confidence interval contained the integer 0; therefore, it can be concluded that no significant bias was observed between the methods evaluated in this study. The correlation coefficient between the automated MPN and the manual method was found to be high (0.953).

Significance: The automated MPN system provides an accurate and easy-to-use approach for the enumeration of total aerobic counts in food contact surface samples. The automated system eliminates the need for media preparation, serial dilutions, manual counting and visual interpretation of results. This, in turn, provides rapid results with the additional advantages of significant economic labor savings, increased efficiency and better traceability.

P1-12 A Multiplex Real-Time PCR Method for Simultaneous Detection of *Salmonella* spp., *Escherichia coli* O157 and *Listeria monocytogenes* in Soft Cheese

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Introduction: Illnesses due to the ingestion of bacterial pathogens in contaminated food cause enormous cost to our nation both medically and economically. Hence, it is important to detect the specific pathogens in contaminated foods that cause diseases such as gastroenteritis, listeriosis, hemolytic uremic syndrome, etc. Availability of a rapid, accurate and sensitive method to detect these pathogens will immensely help FDA to carry out its regulatory mission in a time-sensitive fashion. In this context, there is a need for a rapid and simultaneous detection assay for *Salmonella* spp, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in foods. PCR-based methods for *E. coli*, *Salmonella* and *Listeria monocytogenes*have been reported. However, a universal selective enrichment medium for simultaneous enrichment and detection of these pathogens from various foods pose challenges.

Purpose: To develop a multiplex real-time PCR method for simultaneous detection of *Salmonella* spp., E. *coli* O157 and *Listeria monocytogenes*in soft cheese.

Methods: Soft cheese (Brie) in a previously described selective medium was spiked with ~330 CFU/g of *Salmonella*, *E.coli* O157:H7 and *L. monocytogenes*, stomached and incubated for 2 hours at 37 °C followed by the addition of nalidixic acid, fosfomycin, cycloheximide and acriflavine and grown overnight. The culture was then centrifuged; the pellet was resuspended in 0.25 ml of buffer, boiled for 15 minutes and sonicated. The sonicated sample was centrifuged and the supernatant was used as a template to carry out the multiplex qPCR with primers and taqMan probes targeting *invA* (*Salmonella*), *rfbE* (*E. coli* O157) and *hlyA* (*L. monocytogenes*).

Results: After enrichment, all three gene targets of the pathogens have been detected in the cheese spiked with ~330 CFU/g. The ratio of individual concentration of the four antibiotics used had an effect on the sensitivity of the detection of each pathogen.

Significance: The data suggest that all three pathogens can be simultaneously detected in cheese. This method has the potential to be widely used to simultaneously screen for the presence of *Salmonella*, *E. coli* O157 and *L. monocytogenes*in soft cheese and may be extended to other commodities.

P1-13 Evaluation of a Compact Dry Plate Method for Enumeration of Yeasts and Molds in Foods during a Microval EN ISO 16140 Validation

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Introduction: Compact Dry (Nissui Pharmaceutical Co. Ltd supplied by Hyserve Gmbh & Co. KG) are ready-to-use dry media sheets comprising culture medium, soluble gel and chromogenic compounds. The sheets are rehydrated by inoculating them with 1 ml of diluted food. This study presents the results from comparing Compact Dry YM for yeasts and molds against the standard ISO method in a range of foods.

Purpose: Comparison of Compact Dry YM against the reference method (ISO 21527-1:2008) for enumeration of yeasts and molds using validation protocol EN ISO 16140.

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Methods: Compact Dry YM was used to enumerate numbers of yeasts and molds present in 1 ml samples of diluted foods. The inoculate YM plates were incubated at 25°C for 3-7 days. The standard ISO method used 0.1 ml spread plates on DRBCA incubated at 25°C for up to 5 days. For the expert laboratory study, five inoculation levels in five food types were analysed by both methods; foods tested were meat products, fruit and vegetable products, dairy products, bakery products and mayonnaise. The interlaboratory study involved 9 laboratories in 5 countries. The results were analysed using the principles of EN ISO 16140.

Results: The correlation coefficient (R^2) for all foods was 0.98. The interlaboratory study revealed no evidence of any differences in reproducibility in all levels or repeatability at the medium and high levels between the two methods. The selectivity (inclusivity/exclusivity) of YM was equal to the reference method. Compact Dry YM was found to be equivalent to the standard ISO method for enumeration of yeasts and mold in all foods with a water activity > 0.95.

Significance: Compact Dry YM gave comparable results to the reference method in a convenient ready-to-use format.

P1-14 Evaluation of a Compact Dry Plate Method for Enumeration of *Staphylococcus aureus* in Foods during a Joint MicroVal, AOAC Research Institute EN ISO 16140 Validation

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Introduction: Compact Dry (Nissui Pharmaceutical Co. Ltd. supplied by Hyserve Gmbh & Co. KG) are ready-to-use dry media sheets comprising culture medium, soluble gel and chromogenic compounds. The sheets are rehydrated by inoculating them with 1 ml of diluted food. This study presents the results from comparing Compact Dry X-SA for *Staphylococcus aureus* against the standard ISO method in a range of foods.

Purpose: Comparison of Compact Dry X-SA against the reference method (EN ISO 6888-1:1999) for enumeration of *Staphylococcus aureus*using validation protocol ISO 16140.

Methods: Compact Dry X-SA was used to enumerate numbers of *Staphylococcus aureus* present in 1 ml samples of diluted foods. Inoculated X-SA plates were incubated at 37°C for 24 h and typical blue colonies counted. No confirmation tests were required for this method. The standard ISO method used 0.1 ml spread plates on Baird Parker Agar incubated at 37°C for 24 and 48 hrs. Confirmation using rabbit plasma with EDTA was required. For the expert laboratory study, five inoculation levels in five food types were analyzed by both methods; foods tested were meat products, fish and seafoods, dairy products, bakery products and pasta. The interlaboratory study involved 10 laboratories in 5 countries. The results were analyzed using the principles of EN ISO 16140.

Results: The correlation coefficient (R^2) for all foods was 0.99 and the selectivity (inclusivity/exclusivity) of X-SA was equal to the reference method. Specific AOAC RI analysis (ANOVA, lot-to-lot stability and ruggedness) showed no significant differences between the methods and the interlaboratory study revealed no differences in reproducibility and repeatability between the two methods. Compact Dry X-SA was therefore found to be equivalent to the standard ISO method for enumeration of *Staphylococcus aureus* in all foods.

Significance: Compact Dry gave comparable results to the reference method in a shorter time period without the necessity for confirmation steps.

P1-15 Loop-mediated Isothermal Amplification Assays for Detecting Seven Major Serogroups of Shiga Toxin-producing *Escherichia coli* in Produce

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157 and six other serogroups (O26, O45, O103, O111, O121 and O145) account for the majority of STEC infections in the United States. Produce has been identified as an important transmission vehicle in foodborne outbreaks of the STEC infections.

Purpose: Two sets of loop-mediated isothermal amplification (LAMP) assays, (serogroup-independent and serogroup-specific) were evaluated for the detection of the seven major STEC serogroups in produce samples.

Methods: Lettuce, spinach and sprouts were surface-inoculated with low levels (1-10 CFU/25 g) of STEC strains of the major serogroups. The LAMP assays were used to detect the STEC strains in the produce samples after culture enrichment. Real-time quantitative PCR (qPCR) assays were performed in parallel as a comparison.

Results: The detection limits of the LAMP assays for the STEC ranged from 1 to 20 cells. No false-positive or false-negative results were generated when testing 120 bacterial strains. Sample preparations using centrifugation were efficient in extracting DNA template for LAMP amplifications. LAMP produced positive results in spiked samples in approximately 20 to 45 min after 6 h culture enrichment, whereas pPCR required 35 to 70 min after 6-8 h enrichment to produce the results in the same samples.

Significance: The LAMP assays were able to rapidly detect the seven major STEC serogroups in produce samples with excellent specificity and sensitivity, which could facilitate timely implementation of control measures concerning potential STEC contaminations in produce.

P1-16 FDA *Campylobacter jejuni* and *Campylobacter coli* Detection Method from Raw Silo Milk

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Developing Scientist Competitor

Introduction: *Campylobacter* is recognized as one of the leading foodborne sources of gastroenteritis. The current FDA BAM *Campylobacter* detection method has not been updated since 2001 despite advances made by the scientific community.

Purpose: The purpose of this study was to design and then validate a new detection protocol using current media and molecular techniques against the reference FDA method.

Methods: Pure cultures of ten different strains of *Campylobacter (jejuni & coli)* and five cultures of non-*Campylobacter* strains were serially diluted and inoculated at different levels (5, 50,125 CFU/25g) into separate 25g samples of raw silo milk. Each sample was then enriched in 100 ml of Bolton Broth without blood and incubated in microaerophilic conditions at 41-42°C for 24 hrs. For each sample, 10 µl was streaked in duplicate onto R & F® *Campylobacter* Chromogenic Plating Medium (CCPM) and modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA) which was then incubated in microaerophilic conditions at 41-42°C for 48 hrs. Confirmation was conducted by real-time PCR (qPCR) with a Cepheid Smartcycler utilizing primers and probes (FAM-cj and TxR-cc) within the *ceuE* gene of *Campylobacter*.

Results: At the lowest spiking level (5 cfu/25g), 100% cells were recovered with CCPM compared to 0% with mCCDA. At the low spiking level (50 cfu/25g), the results showed 100% recovery with CCPM compared to 20% with mCCDA. At the high spiking level (125 cfu/25g), both CCPM and mCCDA recovered 100% cells. Confirmation, using a qPCR, verified all the positive isolates as either *C. jejuni* or *coli.*

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Significance: In conclusion, CCPM is a more viable choice for detection at low levels and our qPCR protocol is a demonstrated confirmation step for *C. jejuni* and *coli*. More research with a larger sample size is needed to determine the significant differences for recovering low levels of *Campylobacter* from complex food matrices.

P1-17 Recovery of *Listeria monocytogenes* in RTE Foods Using a 125 g Sample Compared to the 25 g Reference Method

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Introduction: The Food Safety Inspection Service (FSIS) performed a study to determine if RTE meat and poultry products could be analyzed for *Listeria monocytogenes* (*Lm*) in a single, 125 g test portion compared to the 2 5g test portion reference method.

Purpose: To increase *Lm* product test portion size for certain sampling projects in order to enhance public health protection and achieve consistency with *Codex Alimentarius Lm*testing guidelines. This also will permit sample compositing.

Methods: The Midwestern Laboratory spiked samples of cooked turkey with *Lm* at fractional recovery levels. Five sample sets were used, each containing equal numbers of 25 g and 125 g samples spiked randomly. Ninety samples were analyzed at each weight. Each sample was analyzed individually after 20, 22, and 24 hours of primary enrichment incubation using Microbiological Lab Guidebook methodology. All samples were analyzed both culturally and by PCR screen. Reference method samples with fractional recovery between 20% and 80% constituted a valid set.

Results: Of the ninety 125 g samples, 27, 37 and 42 were positive at 20, 22 and 24 hours, respectively, whereas 36 of the 25g samples were positive at all 3 time points. Differences in the proportions of positive samples for 25 g vs. 125 g at 24 h were not significant (Pearson Chi-Square, 2-sided, $P = 0.37$). At 22 hours, a statistically significant difference in the proportions of detected positive samples for 25 g (36/36) vs. that for 125 g (37/42) was observed (Fisher's exact test, 1–sided, $P = 0.04$).

Significance: Five 25 g RTE samples can be composited into a single 125 g sample with little loss of recovery, using a slightly longer incubation time. This allows FSIS to demonstrate method equivalency to its trading partners. Sample compositing allows for significant savings of laboratory resources without compromising method sensitivity. This provides an opportunity to detect more *Lm*-contaminated product.

P1-18 Comparison of a Reference Method of Bioaerosol Sampling to a Newly Developed Compressed Air Microbial Testing Unit (CAMTU)

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Introduction: Compressed air is used in a variety of processes in the food industry. Currently, food manufacturers should validate the safety of all ingredients or processes for regulatory compliance, but unfortunately, there is currently no standard method to evaluate the microbial content of compressed air.

Purpose: The objective was to compare the newly developed Compressed Air Microbial Testing Unit (CAMTU) to a reference Andersen single stage viable particle sizing sampler for recovery of aerosolized *Micrococcus luteus* in compressed air.

Methods: *Micrococcus luteus* ATCC 4698 was used in this study. A biological aerosol was generated using a high pressure nebulizer with 310 kPa air pressure. The biological aerosol within the compressed air was decompressed in a sampling box containing two Andersen single stage viable particle sizing samplers which collected airborne bacteria at a flow rate of 28.3 l/min onto trypticase soy agar (TSA). To test the CAMTU system, compressed air was directly sampled at a flow rate of 46 l/min onto TSA.

Results: Various volumes of air (15, 30, 60 or 121 l) were collected using the Andersen impactor and the compressed air was found to be contaminated at the level of 0.2-0.4 CFU/l. Similar volumes were collected directly into the CAMTU units and aerosolized bacteria were recovered in the range 0.5 -2.5 CFU/l. Low numbers bacteria isolated with both air sampling methods, indicating a large reduction (7-8 log) due to nebulization stress. Despite the low levels in the aerosol, the results show that the CAMTU devise was able to isolate similar cell numbers to the Andersen impactor without a decompression chamber.

Significance: The CAMTU device has been shown to have similar bacterial recovery to the Andersen impactor method. The CAMTU device is a portable, fast testing method for direct sampling compressed air in the food processing environment.

P1-19 Effect of Contamination Matrices on the Persistence of *Escherichia coli* O157:H7 on Romaine Lettuce Leaves

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Introduction: Produce outbreaks that are ultimately traced to pre-harvest farming environments are frequently associated with either direct or indirect contact with animal manure. Despite the numerous matrices found on farm in which foodborne pathogens can survive and in some cases thrive (e.g., compost, manure, soil, water, produce extracts), much sanitizer and surfactant efficacy research is still being performed using either water or pH-buffered saline as the inoculation matrix. This discrepancy between real-world contamination scenarios and laboratory practices has, in some cases, led to the selection of ineffective sanitizing solutions in post-harvest processing of leafy greens.

Purpose: This study examined the effect of growing (and inoculating) *E. coli*O157:H7 in various matrices on its persistence on Romaine lettuce leaves during plant growth and resistance to post-harvest processing.

Methods: *Escherichia coli* O157:H7 cells were inoculated into various matrices (irrigation water, soil, cattle manure or produce extracts). Inocula matrices were applied to the leaves of Romaine lettuce grown in BSL-2 growth chambers at 0, 2, 4, 8, and 16 days prior to harvesting to simulate contamination occurring during produce growing. Lettuce leaves were harvested, stored for 0 to 3 days at 5°C to simulate pre-processing storage, then exposed via simulated commercial washing to various disinfection treatments. Microbial enumeration before and after treatments included use of standard spread-plating and MPN methodologies.

Results: The contamination matrix had a significant impact on pathogen retention and subsequent removal/inactivation. A 4-log reduction of *E. coli* O157:H7 was achieved when the source was a contaminated irrigation water matrix. Lower, 2-log reductions were determined for dairy manure, dairy manure extract and soil which all showed variable responses to chlorine wash reductions, depending on leaf-surface morphology and hydrophobicity.

Significance: Sanitization efficacy tests should be conducted with inoculation matrices mimicking real-life contamination scenarios.

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P1-20 Same-day Detection of *Escherichia coli* O157:H7 and *Salmonella* in 375 g of Ground Beef

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Introduction: A method associating a DNA purification/concentration method and a FRET-based PCR has been evaluated for the detection of *E.coli* O157:H7 and *Salmonella* in 375 g ground beef samples after short enrichment times (6 h and 8 h).

Purpose: The purpose of this study is to reduce the turn around time of food pathogens detection methods. The benefits of a sample preparation containing a DNA concentration/purification method has been evaluated in association with FRET-based specific real-time PCR, which associate the specificity of both primers and probes sequences and the probe melting.

Methods: 375 g of ground beef were inoculated at a low level (target 1-5 CFU/375g) with *E. coli* O157:H7 or with *Salmonella*. After a 48 h equilibration at 4°C, samples were incubated in BPW at 42°C for various times and then analyzed by automated lysis/nucleic acid purification using the Nuclisens easyMAG™ instrument (bioMérieux) followed by a FRET-based real-time PCR. Inoculations were confirmed positive or negative with AOAC approved alternative methods. ***Results:*** Introduction of this simplified nucleic acids purification step upstream of PCR amplification enables to reduce the enrichment step by several hours; this protocol allows detection of *E. coli* O157:H7 after a 6-h enrichment and *Salmonella* after a 8-h enrichment in 100% of the confirmed positive samples.

Significance: This study suggests that the use of a very simple and automated nucleic acid purification step (Nuclisens easyMAG™) allows a turn around time of analysis of 7 h 30 min for *E. coli* O.157:H7 and 9 h 30 min for *Salmonella* sp. Same-day results for both *E. coli* O157:H7 and *Salmonella* in pooled samples enables rapid product release and quicker raw material orientation and therefore cost savings for food producers.

P1-21 16S rDNA Intervening Sequences of Faecalibacterium-like Bacteria: Potential Genetic Markers for Tracking the Source of Fecal Contamination in Food

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Introduction: Fecal coliforms, *Escherichia coli,* and enterococci are widely used as fecal indicator bacteria (FIB) in food. However, the presence of the FIB provides no information of the source of fecal contamination. Knowing the source is critical for taking effective steps to prevent outbreaks of illness associated with consumption of feces-contaminated food and water.

Purpose: This study is to identify host-specific genetic markers within 16S rDNAs of *Faecalibacterium*-like bacteria and to evaluate their uses in tracking the source of fecal contamination in food.

Methods: To identify host-specific genetic markers, a collection of over 20,000 16S rDNA sequences of *Faecalibacterium*-like bacteria, reportedly associated with different host species, was obtained from the Ribosomal Database Project and subject to the comparative analysis via bioinformatics methods, including phylogenic assay and multiple alignments. Based on the newly identified and potentially host-specific genetic markers, polymerase reaction assays (PCR) were developed for the evaluation of each genetic marker's host specificity, using the pooled fecal samples from different animal hosts (dairy cattle, beef cattle, chicken, dog, horse, human, goose, pig, sheep, and turkey) .

Results: Five intervening sequences (IVSs) within the variable region 1 of 16S rDNAs, associated with different *Faecalibacterium*-like bacteria, have been identified by bioinformatics analysis and appear to be host specific as demonstrated by the PCR assays. The IVS-1, -2, and -3 were found to present only in poultry (chicken and turkey) feces, while the IVS-4 and -5 only in cattle and pig feces. In addition, two genetic markers, specific for horse and dog feces respectively, were accidently identified by this study.

Significance: This is the first study on the host specificity of commensal bacteria's ribosomal IVSs, which may prove to be useful genetic markers for identification of the source of fecal contamination in food.

P1-22 Validation of Two New Real-Time PCR Multiplex Kits for the Detection of Shiga Toxin-producing *Escherichia coli* (STEC)

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are pathogens causing severe foodborne illnesses. Although *E. coli* O157:H7 strain was first declared as an adulterant in raw beef products, the six non-O157 STEC, O26, O111, O103, O121, O45, and O145 were recently recognized by the Centers for Disease Control and Prevention as being responsible for nearly 2/3 of all STEC infections, leading them to be declared as adulterants in beef by the United States Department of Agriculture. This USDA regulation is to be enforced starting June 4, 2012.

Purpose: The purpose of this study was to validate two real-time PCR multiplex kits for the detection of the top 6 non-O157 STEC against the USDA MLG 5B.01 reference method.

Methods: The two real-time PCR kits form a complete solution in compliance with the MLG 5B.01 recommendations. iQ-Check STEC VirX allows DNA extraction from bacterial enrichment and detection of the *stx* and *eae* virulence genes. iQ-Check STEC SerO can then be used to screen specifically for the presence of the 6 non-O157 serogroups plus O157:H7. Inclusivity/exclusivity of the method was tested with 230 *E. coli* strains of 165 different O-groups, and 31 non-*E. coli*strains. The sensitivity of the iQ-Check STEC method was compared to that of the MLG 5B.01 method on artificially contaminated matrices. Beef trim samples (375g or 325g) were spiked with low levels (2-10 CFU) of an STEC strain and stored at 4°C for 48h. Samples were subsequently analysed in parallel by both methods, A fractional recovery study, involving 50 samples was carried out for comparison of detection limits.

Results: Presence or absence of *stx* (except for the *stx2f* subtype), *eae*, and top 6 serogroups were correctly determined for all 230 strains tested. There was no significant difference in the performance of the iQ-Check method when compared to the MLG 5B.01 reference method for detection of low levels of STEC in beef trim. The fractional recovery study demonstrated a comparable sensitivity.

Significance: Specificity and sensitivity of the iQ-Check STEC method are equivalent to that of the reference method. It is a reliable and easy-to-use alternative with a reduced time-to-result.

P1-23 Evaluation of a Real-Time PCR Method to Detect *Salmonella* Enteritidis in Whole Shell Eggs

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Introduction: Beginning in July of 2010, the U.S. Food and Drug Administration mandated routine environmental testing of poultry houses for presence of *Salmonella* Enteritidis. If SE is detected in the environment, then eggs must be tested prior to their distribution for sale.

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Purpose: A new and rapid real-time PCR method was developed and evaluated against the FDA BAM culture method for detection of *Salmonella* Enteritidis in whole shell eggs.

Methods: Approximately 250 eggs were combined into a bulk sample for the uninoculated control, and a bulk lot consisting of approximately 900 eggs were inoculated with SE (ATCC 13706) at a concentration of 0.2-2 CFU per 1,000 g. Egg pools consisting of 20 eggs (1,000 g) were prepared. Twenty inoculated and 5 uninoculated egg pools were enriched according to FDA BAM. A second set of 20 inoculated and 5 uninoculated egg pools were supplemented with 100 ml of 10X TSB per pool, and then incubated at 35°C for 24 hours. Real-time PCR was performed on the 7500 Fast using standard conditions (95°C for 10 min; 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds).

Results: Methods comparison showed that the real-time PCR method was equivalent to the FDA BAM method. For two independent studies, chi-square was 0 and 0.41. In addition, the two methods were compared on samples enriched according to the FDA BAM method on day 5 following overnight enrichment in TSB plus ferrous sulfate. Chi-square analysis indicated no difference between the two methods ($\chi^2 = 0$ on two independent studies). No false-positive or false-negative results were observed using either enrichment method. Sample preparation was designed to be completely automated to give results in approximately 3 hours post enrichment.

Significance: The assay evaluated here was shown to be equivalent in performance to the FDA BAM culture procedure for the detection of *Salmonella* in eggs. Real-time PCR detection of SE in whole egg pools is simple, rapid, and more cost effective than the standard FDA BAM culture method.

P1-24 Utilization of Pre-enrichment for Improved Sensitivity of an ELISA-based Detection System for *Escherichia coli* O157:H7

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Introduction: Along with *Escherichia coli*O157:H7, emerging non-O157 strains (O26, O103, O121, O145, O111, and O45) are now recognized by the Centers for Disease Control and Prevention as major contributors of severe foodborne illness. Recent outbreaks associated with emerging non-O157 strains have heightened the need for their enhanced surveillance. For field deployment, a rapid, robust, sensitive, and user-friendly portable assay for these emerging pathogens is much needed.

Purpose: This study evaluated the utilization of a pre-enrichment step for increased sensitivity in an ELISA-based detection system, using commercially available antibodies against *E. coli* O157:H7 for rapid detection in foods in a portable format.

Methods: Overnight bacterial cultures (n=10) were ten-fold serially diluted in pre-enrichment broth (Universal Pre-enrichment broth) and incubated for 6 h at 37°C prior to analysis with a standard sandwich ELISA. *E. coli* isolates comprised both O157:H7 isolates (n=4) and non-O157 isolates (n=6), that were evaluated in triplicate with 3 biological replicates. Lowest detection limit of the assay was determined.

Results: After pre-enrichment, this ELISA assay showed increased sensitivity by two to four-fold from 5.0×10^4 colony forming units (CFU)/ml to 3.0×10^1 CFU/ml for 2 *E. coli* O157:H7 isolates. Two *E. coli* O157:H7 isolates were detected at 4.5×10^2 CFU/ml and 3.80×10^3 CFU/ml. Three of the 5 non-O157:H7 isolates (O26:H11, O103:H11, and O45:H2) showed detection limits ranging from 3.2×10^2 to 72.9×10^3 CFU/ml, and the other three isolates (O145, O111, and O12) were not detected.

Significance: These data suggest that pre-enrichment of samples prior to detection with an ELISA-based system is necessary for adequate/improved detection of *E. coli* O157:H7. Further evaluation and development of these assay components in a lateral-flow luminometer system is ongoing. This simplistic system has potential to further improve the detection sensitivity and specificity for *E. coli* O157:H7 and the emerging non-O157:H7 strains.

P1-25 Evaluation of the BAM EHEC qPCR Assay Results in the ORA Laboratories

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Introduction: The BAM multiplex qPCR assay is designed to detect the *stx*1, *stx*2 genes and the *Escherichia coli* O157:H7 specific +93 SNP of the *uid*A gene. This assay is the first qPCR assay to be routinely used in FDA for regulatory analyses. Questions and concerns have arisen as implementation in the field laboratories has occurred. Performance of the assay in the hands of the field analysts needs to be evaluated so that strengths and limitations of the assay can be assessed.

Purpose: The purpose of this study is to evaluate performance characteristics of the EHEC qPCR screening assay for regulatory use.

Methods: The qPCR runs between August 2007 and September 2010 were collected for evaluation. Data were compiled along with additional information about each sample and sub-sample collected from FACTS and ORADSS. Descriptive statistics and further sub-analyses including Ct values for positive screens and internal amplification controls (IAC) were calculated by laboratory, by program, and by food types. The qPCR runs were interpreted using PCR positive and negative template controls and IAC as the indices to one of three potential qualitative outcomes: negative, cannot rule out (CRO), or invalid.

Results: One thousand and sixty three qPCR runs representing 2,163 samples (16,177 sub-samples) were collected. Twelve samples were confirmed as positive culturally among 300 target positive CROs. One CRO sub-sample was confirmed to contain *E. coli* O157:H7; however, the *uid*A target was not detected in the initial screening. Routine regulatory use of the qPCR screening assay for *E. coli* O157:H7 was capable of ruling out 82.3% of tested sub-samples as negative within 24 hours. The inconclusive CRO results (12.7%) may be caused by high generic *E. coli* background, reagent lot, human error, or possible matrix inhibition.

Significance: The data suggest that actions such as training, adequate DNA decontamination and modified procedure are recommended to improve the performance of EHEC qPCR assay.

P1-26 FERN Multi-laboratory Evaluation of MicroSEQ® *Salmonella* spp. Detection Kit in Comparison with an FDA Rapid Screening qPCR Method

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Introduction: *Salmonella* spp. are the most frequently reported cause of foodborne illness worldwide. To augment time-consuming conventional culture methods, the FDA developed a qPCR method for rapid screening purposes. This method has been shown to be reliable and accurate and has been validated by the FERN (Food Emergency Response Network) in a multi-laboratory validation study using both ABI 7500 FAST and SmartCycler II systems. The method is used routinely by the FERN and in FDA mobile laboratory deployments. To expand the repertoire of available molecular diagnostic tests for *Salmonella* in foods, the FERN has conducted a similar multi-laboratory validation study to evaluate the performance of the MicroSEQ® *Salmonella spp.* Detection Kit (Life Technologies, Inc.).

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Purpose: To compare the sensitivity and specificity of the MicroSEQ® *Salmonella* spp. to those performance parameters previously validated for the FDA qPCR rapid screening method for *Salmonella*.

Methods: Four food types (chili powder, soft cheese, fish and tomatoes) were inoculated at three levels (six replicates for each) – uninoculated, low (1-5 CFU/25g) and high (10-50 CFU/25g). All samples were tested for *Salmonella* using the 24-hr qPCR method which utilizes modified Buffered Peptone Water (mBPW) as the sole enrichment medium. Eighteen samples for each food type were independently analyzed by the participating laboratories using the MicroSEQ® *Salmonella*spp. Detection Kit in parallel with the qPCR method using both ABI 7500 FAST and SmartCycler II systems.

Results: For all food types, the sensitivity is 1/288 for FDA qPCR and 2/288 for MicroSEQ®. The selectivity is 513/576 for FDA qPCR and 515/576 for MicroSEQ® indicating that there was no significant difference ($P \geq 0.05$) statistically between the MicroSEQ® *Salmonella* spp. Detection Kit method and the corresponding reference methods.

Significance: The consistent results among 12 laboratories support the utility of the MicroSEQ® *Salmonella spp.* Detection Kit as an alternative method for detecting *Salmonella* in food.

P1-27 Recovery of *Escherichia coli* O157:H7 from Ground Beef after Enrichment in Three Different Media Types Using the BAX® Real-Time PCR Assay

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Introduction: The Provincial Laboratory for Public Health, Alberta, works with local health inspectors to investigate foodborne illness investigations. It is essential to identify *Escherichia coli* O157:H7 from food samples with high background contamination.

Purpose: This study evaluated the BAX® Q7 Real-Time PCR Assay for *Escherichia coli*O157:H7 after enrichment in three different enrichment media.

Methods: A panel of 12 human clinical *Escherichia coli* O157:H7 isolates (inclusivity) and 16 non-*E. coli* O157:H7 isolates (exclusivity) were tested against mTSB-CCV (cefixime, cefsulodin, and vancomycin), mTSB-n (novobiocin), and BAX MP commercial broth at a concentration of 100 CFU/250ml. To determine sensitivities, four *E. coli* O157:H7 isolates were spiked into raw ground beef at 100, 10, and 1 CFU/25g and enriched in each media. All results were confirmed by culture using the Health Canada reference culture method (MFLP-80), and extent of background flora was estimated.

Results: The BAX® assay detected 100% (25/25) of the samples in the inclusivity panel and none in the exclusivity panel. A 100% positivity rate was observed when samples were inoculated at 100 and 10 CFU/25g but dropped to 63.5% (94/148) when the inoculum was 1 CFU/25g. A low false negative rate was seen due to a single sample inoculated at 1 CFU/25g. Enrichment in mTSB-CCV significantly reduced background growth within the matrix ($P = 0.0012$; Fischer's Exact Test); however mTSB-n and BAX MP were more likely to yield a positive result ($P < 0.0001$; $P = 0.0294$) when background flora in the matrix was high.

Significance: The BAX® assay demonstrated a high degree of specificity towards *Escherichia coli* O157:H7. Although media containing CCV was more likely to reduce the background flora in the ground beef matrix after enrichment, use of mTSB-n or BAX MP media may lead to improved detection of *E. coli* O157:H7 in foods with high background contamination.

P1-28 Comparison of Different Enrichment Media for Non-O157 Shiga Toxin-producing *Escherichia coli* Strains in Beef Trim

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) strains other than serotype O157 represent some of the most important causes of foodborne infections worldwide. Detection methods for STEC in foods include culture enrichments followed by molecular, immunological and cultural characterization.

Purpose: To compare different culture media for enriching the "top 6" non-O157 STEC serotypes.

Methods: Two to fourteen CFU of each serotype (O26, O45, O103, O111, O121 and O145) were spiked into 375 g of beef trim, stored overnight at 4°C, and then enriched with five different media: IEH medium (A), LES medium (B), mTSB (C), mTSB+novobiocin (D), and mTSB+vancomycin (E). Sample aliquots were taken after 9, 12 and 20 h incubation at 42°C, and analyzed using an in-house, non-O157 STEC detection system that included multiplex PCR and lateral flow immunoassay (LFI).

Results: As early as after 9 h of incubation in Media A, B, C and E, most of the non-O157 serotypes were detected by multiplex PCR. All six non-O157 serotypes were detected in beef trim by using both multiplex PCR and LFI after the 12 h incubation. After enrichment in Medium D (the USDA-FSIS' medium formulation for STEC containing novobiocin as selective inhibitor) produced positive PCR signals only after 12 and 20 h of incubation, and completely inhibited the growth of the O111 serotype. Growth of the O111 serotype in mTSB supplemented with different concentrations of novobiocin (mTSB) showed that as little as 5 ug/ml delayed the growth of the O111 serotype.

Significance: With the exception of the novobiocin-supplemented medium, the culture media used in this study effectively allowed enrichment of the non-O157 STEC strains artificially inoculated in beef trim and therefore could be used as an alternative to the most commonly used enrichment medium for the six non-O157 STEC serotypes.

P1-29 Development and Validation of a Real-Time PCR Method for Detecting Non-O157 Shiga Toxin-producing *Escherichia coli* Strains in Beef

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) strains other than serotype O157 represents some of the most important causes of foodborne infections worldwide, but their occurrence is probably underestimated because of the lack of detection methods for these serotypes.

Purpose: To develop a real-time PCR method for detecting the "top six" non-O157 STEC serotypes, and to validate its performance on artificially inoculated beef trim.

Methods: Real-time PCR primers and probes specific for the non-O157 STEC were designed and the PCR conditions were optimized. About 1 to 8 CFU of each serotype (O26, O45, O103, O111, O121 and O145) were spiked into 375 g of beef trim, stored overnight at 4°C, and then enriched with mTSB + 8 ug/ml vancomycin. Sample aliquots were taken after 12 h incubation at 42°C, and analyzed using real-time PCR. **Results** were confirmed using the USDA-FSIS MLG 5B.01 method.

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Results: A multiplex real-time PCR assay for screening and detecting non-O157 STEC was developed and validated, using an intra-laboratory validation procedure based on the USDA FSIS MLG 5B.01 method. The detection limit of the real-time PCR method was 10^4 cells/ml and was determined by using cell dilutions in triplicate assays. The performance of the assay was assessed by using on 20 replicates of artificially inoculated beef samples and compared with the MLG 5B.01 method. The real-time PCR method gave 100% concordance with the MLG 5B.01 method. The developed real-time PCR assay detected non-O157 STEC at a level of ~1 to 8 CFU per 375 g beef samples after 12 h enrichment in modified tryptic soy broth (mTSB) supplemented with vancomycin.

Significance: This real-time PCR assay in combination with the modified enrichment medium effectively detects non-O157 STEC from spiked meat samples at a shorter incubation time than the USDA-FSIS MLG 5B.01 method.

P1-30 Simple Filter Paper as a Shipping and Storage Medium for Human Enteric Viruses

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Developing Scientist Competitor

Introduction: Over 100 types of pathogenic enteric viruses have been found in sewage-contaminated drinking water environments. Sample collection from remote environmental sites can be problematic due to the need for an uninterrupted cold chain to guarantee sample suitability for molecular detection.

Purpose: The purpose of this work was to investigate the feasibility of using filter paper as an easy and inexpensive method to collect environmental samples for enteric virus testing, as an alternative to refrigerated transport and storage.

Methods: Different concentrations (10^3 to 10^6 PFU/ml) of two enteric virus surrogates (F-RNA coliphages MS2 and Qbeta) diluted in buffer or a 10% bovine manure slurry were maintained at 4°C for 37 days. Ten µl aliquots of the dilutions were also spotted on individual 6 mm diameter filter paper (Whatman No. 1) circles to give final amounts of 10^1 to 10^4PFU, dried and stored at 37°C for 37 days. Triplicate samples of the liquid (10 µl) or disk samples taken at 0, 6, 13 and 37 days were analyzed by real time RT-PCR.

Results: The minimum amount of MS2 or Qbeta detected consistently by RT-PCR in control samples was 10^2 PFU. There was no difference ($P > 0.01$) in detection level on any sampling day for phage MS2 between liquid samples or the filter disk samples for the buffer or manure slurry samples. For Qbeta, the detectable amount of bacteriophage spotted on filter paper at 37°C was on average one log lower (10^3 PFU) than the amount detected in samples stored in liquid form at 4°C. This difference was statistically significant at 0 ($P = 0.0005$) and 37 d ($P = 0.006$) in the buffer samples and at 0 d ($P = 0.0032$) in the manure slurry samples.

Significance: These results suggest that filter paper may be used as a storage and transport method for enteric viruses, providing a sampling alternative when refrigeration of the sample is not possible.

P1-31 Validation of a Test System for Detecting Non-O157 Shiga Toxin-producing *Escherichia coli* Strains in Beef

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) strains other than serotype O157 represents some of the most important causes of foodborne infections worldwide, but their occurrence is probably underestimated because of the lack of an integrated detection method for these serotypes.

Purpose: To validate a test system based on multiplex PCR (mPCR) and lateral flow immunoassay (LFI) for detecting the "top six" non-O157 STEC serotypes, and to evaluate its performance on artificially inoculated beef trim.

Methods: About 3 to 6 CFU of each serotype (O26, O45, O103, O111, O121 and O145) were spiked into 375 g of beef trim, stored overnight at 4°C, and then enriched with mTSB + 8 ug/ml vancomycin. Sample aliquots were taken after 9 and 12 h incubation at 42°C, and analyzed by using multiplex PCR and LFI. **Results** were confirmed using the USDA-FSIS MLG 5B.01 method.

Results: A multiplex PCR assay for screening and detecting non-O157 STEC using intimin, Shiga-toxin, and serotype-specific O-antigen flippase genes as targets, was developed and validated. The detection limit of the multiplex PCR and LFI methods were 10^4 cells/ml and 10^5cells/ml, respectively. The performance of the test system was assessed on 20 replicates of artificially inoculated beef samples and compared with the MLG 5B.01 method. Positive PCR signals were detected as early as after 9 h enrichment, and all confirmed positives were detected after 12 h. Both the multiplex PCR and LFI methods gave 100% concordance with the MLG 5B.01 results.

Significance: The test system effectively detects six non-O157 STEC serotypes from spiked meat samples at a shorter incubation time (12 h) than the MLG 5B.01 method (15-22 h).

P1-32 Potential of Dye Uptake and Interference with qPCR Assays by Pooled STEC Colony Picks

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Introduction: Rapid detection and isolation of Shiga toxin-producing *E. coli* (STEC) is essential to quickly identify a contaminated food product and limit the outbreak of disease. However, once detected in a sample, it is often difficult to isolate STEC from generic *E. coli*. Pooling multiple suspect colonies from L-EMB and applying a qPCR for presence of *stx*genes can quickly rule in/out colonies. However, samples with multiple picks resulted in false positive readings and excessive background fluorescence warnings in the qPCR instrument.

Purpose: This study sought to determine the source of spectral interference of *E. coli* colony picks from L-EMB agar and eliminate this interference while maintaining time-saving benefits of pooling colony picks for screening.

Methods: Varying number (1, 2, 4, 8, 16) of replicate STEC colony picks was pooled, extracted by boiling, and analyzed by a multiplex qPCR (*stx*1, *stx*2, *uid*A SNP). Raw fluorescent data on qPCR instrument was recorded for each sample. Samples were also analyzed by absorbance scans on micro-spectrophotometer.

Results: Increasing number of colony picks resulted in visual pigment as well as instrument recorded increases in fluorescence. Specifically, incremental increases were observed in the emission channel of 565-590 nm. Spectral scans (max abs ≈ 520nm) suggest the eosin component of L-EMB taken up by extracted colonies resulted in interference. Dilution (1:10) reduced this non-specific background fluorescence to achieve acceptable qPCR results.

Significance: Dyes taken up from differential media by bacterial colonies can potentially interfere with qPCR assays that target foodborne pathogens. Simple dilution of sample can alleviate this interference while maintaining gene target sensitivity.

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P1-33 Evaluation of Surface Sampling Performance of Four Commercially Available Swab Materials on Human Norovirus GII.4

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Introduction: A swab rinse method is widely used to determine contamination levels of viruses on environmental surfaces. However, most of these methods have not been evaluated and the sampling performance remains unknown.

Purpose: We evaluated the sampling performance of four different swab materials (cotton, rayon, polyester and macrofoam) on a stainless steel surface under variable test conditions.

Methods: A clarified human GII.4 norovirus (GII.4 NoV) stool suspension ($10^{7.0}$ RNA copies/ml) was spread on stainless steel coupons, dried out at RT, and swabbed with each swab material. Different drying times (1, 8, 24, and 48 hours) and coupon size (4, 9, 16, and 25 inches^2) were tested. Recovered GII.4 NoV was quantified by real time RT-PCR.

Results: The effect of drying time or surface area on sampling performance varied significantly by swab type (P <0.001). When GII.4 NoV RNA ($10^{5.1}$ RNA copies) were seeded on coupons of 9 and 25 inches^2 and sampled after 8 and 48 hours of drying, four swab materials recovered GII.4 NoV (range 1.8 to 8.6%) from 9 inches^2 coupons. However, for the 25 inches^{2}coupons, only macrofoam swabs were able to recover the virus (recovery ≥2.1 %).

Significance: In conclusion, macrofoam swabs showed superior sampling performance compared to cotton, rayon, and polyester swab materials for the recovery of GII.4 NoV from a stainless steel surface. In addition, the ability of macrofoam to recover virus from 25 inches^2 surface areas, makes it a promising tool for the detecting of environmental reservoirs in high-risk NoV settings such as restaurants, hospitals, nursing homes and cruise ships.

P1-34 Comparison of Different Agars for the Recovery and Isolation of Non-O157 STECs from Baby Spinach and a Raw Milk Cheese

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Introduction: The FDA *Bacteriological Analytical Manual* (BAM) recommends Levine's Eosin Methylene Blue (L-EMB) agar for isolating non-O157 Shiga toxin-producing *E. coli* (STEC). However, because this agar cannot distinguish non-pathogenic *E. coli* from STEC, it is necessary to screen large numbers of colonies. Other agars that target *E. coli* O157:H7 have not been thoroughly tested for the recovery of non-O157:H7 STECs.

Purpose: Evaluate the efficacy of different agar formulations for selective recovery of non-O157 STECs from various food matrices.

Methods: Four replicate 25 g test-portions of baby spinach were spiked at levels of approximately 0.02 CFU/g with virulent STEC strains. BAM procedures were followed using 6 agars: Rainbow O157, modified Rainbow O157, R & F *E.coli* O157, CHROMagar, washed blood (SHIBAM), and L-EMB. All agars were also evaluated using a raw-milk cheese purchased in New Mexico and subjected to BAM procedures for the recovery of *E. coli*.

Results: For the spiked studies, the results were strain conditional. For example, all of the agars recovered an O26 strain, while plates from the same batch (Rainbow O157 (modified and unmodified), CHROMagar, and R & F) failed to support the growth and recovery of an O111 strain. When a raw-milk cheese was used for agar evaluation, generic *E. coli* was recovered from all agars. However, the appearance of *E. coli* on both Rainbow O157 agar types varied from pink to blue to purple and required multiple colony picks as compared to the more straightforward color differentiation or hemolysis (SHIBAM agar) offered by the other agars.

Significance: Agar performance was highly strain-dependent and variable colony coloration made picking colonies, based on color, difficult. Follow-up studies evaluating agar performance with additional panels of STEC strains is warranted. Until then, the use of multiple agars (including one without supplements to decrease background microflora) is recommended.

P1-35 Validation of a Commercial Real-Time PCR Assay for Screening *Salmonella* in Foods

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Introduction: *Salmonella* is found in many food and environmental sources and can cause serious illness. Since its isolation is long and difficult when in the presence of competing flora, non-culture, rapid detection methods are needed for this organism. To improve assay performance, a real-time version of the BAX® System *Salmonella* assay was designed, which reduces instrument processing time to approximately one hour.

Purpose: This study evaluated the effectiveness of the test kit for screening *Salmonella*from ground beef, lettuce, chicken, cream cheese, dry pet food, and stainless steel environmental surfaces.

Methods: Artificially contaminated foods and environmental surfaces were tested and results compared with the appropriate Health Canada, USDA or FDA reference culture method(s). Samples were inoculated with *Salmonella* at levels expected to yield fractional positive results based on preparatory studies. All sample types were enriched in the appropriate reference method primary enrichment (LB or BPW). Corresponding replicates were also enriched in an alternative media (BAX®System MP media or TSB with novobiocin) where appropriate to improve method performance. For ground beef testing, the reference method was tested on 25 g analytical portions while the alternative method was tested on 375 g portions (25 g spiked sample combined with 350 g of unspiked material) to reflect industry testing norms. Secondary enrichment and culture confirmation from all enrichments was conducted using the appropriate reference method(s).

Results: Testing included 240 spiked and 60 unspiked samples. For ground beef, lettuce, chicken, and cream cheese, 29/100 spiked reference method enrichments were culture positive while 28/100 spiked test method samples were positive by PCR from the alternative enrichments. For pet food and environmental testing, LB and BPW enrichments were found to be equivalent when testing by culture and by the BAX®System method, with 5/20 spiked pet food samples and 13/20 spiked environmental samples being PCR positive for each enrichment method. All PCR-positive samples culture confirmed and all PCR-negative samples were negative by culture. Statistical analysis revealed no significant difference in the alternative PCR and reference culture methods.

Significance: This study indicates that PCR detection of *Salmonella* using the BAX® System real-time assay is rapid and sensitive. Test kit results demonstrate no significant difference when compared with the reference culture methods.

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P1-36 Growth of *Escherichia coli* O157:H7 in Common Pre-enrichment Broths

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Introduction: Pathogens are often present in foods at low levels and an enrichment step is typically required for detection using cultural or molecular techniques. Antibiotics and increased temperatures are common means of increasing enrichment selectivity.

Purpose: The growth of *Escherichia coli* O157:H7 in brain-heart infusion broth (BHI), buffered peptone water (BPW), BPW with vancomycin (BPW+V) and BPW with vancomycin, cefixme and cefsulodin (BPW+VCC) was compared at different temperatures.

Methods: An overnight culture of *E. coli*O157:H7 in tryptic soy broth was diluted to 3 log CFU/ml in each broth. Four hundred microliters were loaded into each well of a Bioscreen C Honeycomb Plate, with eight inoculated wells and two negative controls for each medium. Plates were incubated in the Bioscreen C at 30, 35 or 41°C for 10-15 hours, with optical density (OD) readings every 15 minutes. Three trials were performed at each temperature and the resulting growth curves were fitted to the Gompertz equation using DMFit.

Results: At 30°C, the growth rates in BHI, BPW, BPW+V and BPW+VCC were 0.291 ± 0.008, 0.051±0.005, 0.046 ± 0.002 and 0.037 ± 0.007 hr^{1}, respectively. At 35°C, these growth rates were 0.349 ± 0.004, 0.057 ± 0.002, 0.053 ± 0.002 and 0.049 ± 0.001 hr^{1}. Compared to BHI, only small increases in OD were observed in the BPW variations at the lower temperatures. At 41°C, the OD did not increase in the BPW-based enrichments, even when incubation was extended to 24 hours. The growth rate in BHI at 41°C was 0.326 ± 0.015 hr^{1}.

Significance: Increased temperatures and antibiotics are commonly used during enrichment for *E. coli* O157:H7. However, no growth was observed in BPW, BPW+V or BPW+VCC at 41°C. These conditions do not appear to be sufficient for the enrichment of this organism. Future experiments with the Bioscreen C will assess the growth of other strains and microorganisms in a variety of enrichment media.

P1-37 Concentration of Spiked *Salmonella* spp. and *Escherichia coli* O157:H7 from Large Volumes of Irrigation Water with Subsequent Detection by the VIDAS Technology

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Developing Scientist Competitor

Introduction: Testing of fresh produce for major foodborne pathogens such as *Salmonella* spp. and *Escherichia coli* O157:H7 is impractical due to small sample sizes, variability of contamination, and low sensitivity of current platforms. Testing of water (irrigation or wash water) offers greater probability for pathogen detection when combined with appropriate sample preparation and concentration methods.

Purpose: The purpose of this study was to evaluate the effectiveness of sample concentration via Moore swabs (MS) or disposable inline filters (DIF) and selective enrichments to detect *Salmonella* spp. and *E. coli* O157:H7 in irrigation water using VIDAS technology.

Methods: Three-strain cocktails of *Salmonella* spp. or *E. coli* O157:H7 strains associated with produce outbreaks were used to contaminate large volumes of irrigation water (10 l) at concentrations of 0.1, 1, 10, and 100 CFU/ml. Samples were enriched pre- and post-concentration in buffered peptone water containing 8 mg/l vancomycin (*E. coli* O157:H7) or *Salmonella* supplement (*Salmonella*) for up to 18 hours at 42°C. Samples were tested at 8, 12, and 18 hours.

Results: Both target pathogens were detected at the lowest spiking level of 0.1 CFU/ml following concentration and enrichment for 8 hours, with a significantly higher (P < 0.001) number of samples being detected following concentration compared to non-concentrated samples. For *E. coli*, detection of the higher contamination levels was possible pre-concentration at 8 hours, but for *Salmonella*, only 100 CFU/ml could be detected pre-concentration after 18 hours of enrichment.

Significance: The results of this study show that the VIDAS technology can be used to sensitively and specifically detect *Salmonella* spp. and *E. coli* O157:H7 in irrigation water. Concentration protocols employed here greatly shortened the time-to-detection and improved sensitivity of the previously established methodology.

P1-38 Detection and Isolation of *Salmonella* from Naturally Contaminated Pine Nuts Using Several Different Preenrichment Media

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Introduction: A multistate outbreak of *Salmonella* Enteritidis infections, linked to Turkish pine nuts purchased from bulk bins at grocery store chain, located in the Mid-Atlantic States, was reported by CDC from October to November 2011. A total of 43 individuals from 5 states were infected with the outbreak strain of *Salmonella* Enteritidis.

Purpose: To determine the relative efficacies of five preenrichment media for the detection of *Salmonella*from naturally contaminated pine nuts: lactose broth (LB), buffered peptone water (BPW), modified BPW (mBPW), universal preenrichment (UP) broth, and BAX broth.

Methods: Twenty-five g pine nut test portions were soaked in 225 ml portions of LB, BPW, mBPW, UP and BAX broths. The preenrichments were incubated for 24 h at 35°C. The *Bacteriological Analytical Method* (BAM) *Salmonella* culture method was followed thereafter. The contamination level was determined with the most probable number method. Two real-time PCR (qPCR) analyses were performed on 24 h-incubated preenrichment media on ABI Fast 7500. qPCR1 is a singleplex PCR assay developed by FDA to detect the *invA* gene (unique to *Salmonella*) and is currently deployed by FDA's mobile lab. qPCR2 is a multiplex qPCR assay recently developed by FDA to detect *Salmonella* by targeting *invA* and *ttr* genes to enhance the reliability of detection results.

Results: The BAM culture results showed no significant differences (P > 0.05) among the five different preenrichment media for the detection of *Salmonella* from pine nuts. However, both qPCR procedures had significantly (P < 0.05) higher false negative rates when used with LB, the current BAM *Salmonella*preenrichment medium for pine nuts, as compared to the other four media. qPCR results, from both qPCR procedures, corresponded perfectly with culture results from mBPW, UP and BAX media (100% sensitivity).

Significance: This study addressed a need to improve current BAM *Salmonella* culture method for the detection of *Salmonella* from pine nuts when using real-time PCR as screening method.

P1-39 Development and Validation of an Immunoaffinity Column for Detection of Aflatoxins in Agricultural Produce

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Introduction: Aflatoxins are recognized as the most important group of mycotoxins. They are naturally synthesized by a few *Aspergillus* species, mainly *A. flavus* and *A. parasiticus*. There are several aflatoxins; types B_1, B_2, G_1 and G_2 are most common. Aflatoxin B_1 is considered the most toxic and carcinogenic substance produced in nature. FDA has established action levels for the amount of aflatoxin allowed in food or feed to protect human and animal health.

Purpose: To develop and validate immunoaffinity columns for accurate and reliable detection of aflatoxin in different food matrixes.

Methods: The immunoaffinity column was prepared by attaching high affinity monoclonal antibodies against aflatoxin to agarose matrix in the column. The optimal amount of antibodies to use and the buffer system were developed. Performance of immunocolumns was evaluated as percent of aflatoxin recovery from spiked food matrices such as corn, peanuts and almonds. Aflatoxin was spiked into the sample extraction solution at concentrations ranging from 2 to 120 ppb, processed and tested by HPLC according to the AOAC Official Method 991.31 (JL Analytical Services Test Method SOP#: 3A00150, Ver. 14). The performance of the immunocolumns was compared with that of the VICAM AflaTest WB SR (the currently AOAC approved product) on spiked as well as field samples.

Results: Immunoaffinity columns were developed for accurate and reliable aflatoxin detection in different food matrices. Based on 35 samples spiked with 8 ppb of aflatoxin, the mean percent recovery of IEH AflaColumns was 90.58%, with a standard deviation of 4.54 and recovery range of 84-100%. This values compared favorably against those obtained when using VICAM AflaTest columns.

Significance: We developed and validated an improved immunoaffinity method for aflatoxin detection in different food matrices.

P1-40 Performance of a New Molecular Platform for the Recovery and Detection of *Salmonella* spp. from Fresh Raspberries

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Introduction: Molecular methods are of interest for the food industry because the benefit of obtaining results in a relatively short time compared with traditional culture methods. The 3M™ Molecular Detection System combines two technologies: Isothermal DNA Amplification, which uses multiple, specific primers targeting distinct regions of the genome, and Bioluminescence real-time detection. The wide variety of food commodities requires proper validation of alternative methods for *Salmonella* detection.

Purpose: The purpose of this study was to compare the recovery of *Salmonella*from inoculated fresh raspberries with the 3M™ Molecular Detection System and traditional culture method.

Methods: *Salmonella* spp. strains isolated from berries farm environments (*S.* Branderup*, S.* Montevideo*, S.* Anatum*, S.* Infantis*, S.* Agona and *S.* Poona*)* were used to inoculate raspberries at 1-5 CFU/25g. Additionally, uninoculated fruit portions were tested. Samples were analyzed according the Food and Drug Administration´s Bacteriological Analytical Manual and 3M Molecular Detection System (MDS) methods. Sample preparation (soak and stomaching) for pre-enrichment step was also evaluated. Data between recovery methods were compared using McNemar's test for paired data at significant level of 0.05.

Results: *Salmonella* spp. were recovered from all soak samples with the 3M™ Molecular Detection System and traditional culture method. All stomached samples yielded negative results by both methods. False-positive results were not obtained.

Significance: The 3M™ Molecular Detection Assay *Salmonella* can be implemented for the suitable recovery of *Salmonella* spp. from raspberries. Homogenization of pre-enrichment broth/sample produces false-negative results with either traditional culture methods or 3M™ Molecular Detection System.

P1-41 Hepatitis E Virus: A New Food Safety Issue?

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Introduction: In recent years, several autochtonous hepatitis E cases and a high seroprevalence have been reported in the U.S. and Europe. These data indicate a high prevalence of hepatitis E virus infections.

Purpose: A potential source of contamination is the consumption of porcine produce or food contaminated by an environmental source. As suggested by the CDC and EFSA, the objective of the study was to evaluate the prevalence of hepatitis E virus (HEV) in food samples, not only evaluating pork produce.

Methods: A global method for HEV detection in environmental or food samples was set up. Based on methods developed for norovirus detection in food samples, standard protocols have been developed and validated. The kit hepatitisE@ceeramTools was used for real time RT-PCR detection. A large prevalence study was then conducted on 440 food samples collected worldwide in food companies in 2011. These samples include pork liver sausages (4), shellfish (36), fruits (77), vegetables (12), herbs and spices (230), process water (62), and ready-to-eat food (20). These samples were also tested for norovirus GI, GII and hepatitis A virus (HAV).

Results: A limit of quantification for the global method of 500 genome copies was obtained whatever the samples. Below this limit, a sample is considered positive but not quantifiable with reliability. The prevalence levels for norovirus GI, GII and HAV were of 2.95%, 8.6% and 0.45%, respectively. The results obtained for HEV demonstrate a prevalence of 0.9% with positive samples including pork liver sausage, pepper and laurel powder.

Significance: To our knowledge, this is the first large study conducted on HEV prevalence in food samples. Our results demonstrate a prevalence for HEV in food samples, in the same range as hepatitis A virus. These results confirm that hepatitis E virus should be included in prevalence studies concerning foodborne virus safety issues.

P1-42 Comparison of Molecular and Classical Serotyping Approaches for Serovar Identification of *Salmonella* Isolated from Food Products

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Introduction: Classical serotyping utilizes antisera to determine agglutination patterns of the O and H antigens; there are > 2,500 *Salmonella* serotypes described by the Kauffman-White (KW) scheme. Challenges associated with classical serotyping include differences in antisera quality, reagent shortages, and reproducibility of results. A molecular serotyping approach would address these issues. PremiTest *Salmonella* (PTS) is a commercial DNA microarray-based typing assay able to identify common serovars of *S. enterica*utilizing a multiplex ligation detection reaction approach.

Purpose: The objective was to assess the utility of PTS as a *Salmonella* serotype identification tool in comparison to traditional serotyping by the KW scheme.

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Methods: 112 *Salmonella* isolates from the Silliker culture collection, including 38 isolates from food products, were used in this study. Samples were prepared for PTS analysis following manufacturer protocol. Twenty blinded samples from routine testing were also screened with the PTS assay; identity of these isolates was determined by traditional serotyping.

Results: The PTS and classical serotyping results matched for 68 isolates (61%); 6 additional isolates were correctly matched after being checked manually. Similarly, 12 of 20 blinded samples matched the traditional serotyping result (60%). Sixteen of 46 isolates that did not match were isolated from a variety of foods (e.g., peanuts, pasta, cheese powder, M&B meal, liquid egg). Six serotypes (Bovismorbificans, Kentucky, Newport, Ohio, Reading, Worthington) showed new pattern codes despite being represented among 100 serotypes in the validated PTS database. Considering the higher discriminatory power of PTS, two or more spot patterns can occur within a conventional serotype.

Significance: Currently, PTS can detect >300 *Salmonella* serotypes and has validated identification of >100 serotypes. However, the majority of patterns in the PTS database have been limited to isolates of European origin. Efforts to expand the database by analyzing isolates from different geographic locations, including strains isolated from food products, will improve robustness and performance of the methodology. An advantage of PTS is that extension of serotypes does not necessarily require a change to the DNA markers on the microarray, but that the score can be improved by simply adding more isolates to the database.

P1-43 A Comparative Evaluation for *Listeria* Species in Food with the Roka *Listeria* Detection Assay on Atlas™ System vs. DuPont Qualicon's BAX® System 24E Assay

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Introduction: *Listeria* is an environmentally ubiquitous organism implicated as a major cause of human foodborne illness worldwide. *Listeria's* long lag phase and hindered growth in the presence of competitive flora presents a challenge for rapid and reliable detection with a single 24-hour enrichment step.

Purpose: Compare the detection and assay efficiency of *Listeria monocytogenes* in inoculated foods after 24-28 hour enrichment with the Roka Bioscience Listeria Detection Assay on Atlas™ System vs. DuPont Qualicon's BAX® System PCR 24E Assay.

Methods: Five foods are assessed: 1) hummus, 2) cantaloupe, 3) frozen waffle, 4) fresh mozzarella, and 5) deli turkey. Five non-inoculated and 20 inoculated samples are tested and compared to culture. For the Roka assay, Half-Fraser is added, incubated at 35°C for 24-28 hours, a sample transferred to a collection tube containing a lysis reagent, and tested on Atlas™ System. For the BAX assay, 24 LEB is added, incubated at 37°C for 24-28 hours, a sample transferred to cluster tubes for a two-part lysis procedure, a PCR tablet is hydrated with lysate, and tested on BAX. Post enrichment enumeration on MOX agar is determined with discrepant analysis utilizing motility and catalase tests.

Results: The Roka assay demonstrates 100% sensitivity and specificity for all five foods compared to culture. The BAX assay demonstrates in hummus and fresh mozzarella 100% sensitivity and specificity, in cantaloupe 93.3% sensitivity and 73.3% specificity, in frozen waffle 100% sensitivity and 78.9% specificity, and in deli turkey 100% sensitivity and 93.8% specificity.

Significance: The Roka assay demonstrates improved sensitivity and specificity compared to the BAX assay in cantaloupe, frozen waffles, deli turkey, and comparable results in hummus and fresh mozzarella after 24-28 hours of enrichment.

P1-44 A Comparative Evaluation of Environmental Samples from Food Plants with the Roka *Listeria* Detection Assay on Atlas™ System, DuPont Qualicon's BAX® System 24E Assay, and bioMerieux VIDAS® LSX

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Introduction: *Listeria* is an environmentally ubiquitous organism implicated as a major cause of human foodborne illness worldwide. *Listeria's* long lag phase and hindered growth in the presence of competitive flora presents a challenge for rapid and reliable detection with a 24 hour enrichment step.

Purpose: Compare the detection and assay efficiency of *Listeria* species in environmental samples after 24-28 hour enrichment with the Roka Listeria Detection Assay on Atlas™ System**,** DuPont Qualicon's BAX® System PCR 24E Assay, and bioMérieux VIDAS® LSX.

Methods: Divide environmental sponges (n=130) into 3 subsamples for parallel analysis as follows: add 30mL Butterfields, transfer 10mL to 3 enrichment bags, perform assay, and compare to culture. For the Roka assay, enrich 24-28 hours in Half-Fraser at 35°C, transfer sample to a collection tube containing lysis reagent, and test on the Atlas System. For the BAX assay, enrich 24-28 hours in 24 LEB at 37°C, transfer sample to cluster tubes for two part lysis procedure, hydrate PCR tablet with lysate, and test on BAX. For the VIDAS assay, enrich 24-26 hours in LX at 30°C, transfer sample to a tube for high temperature lysis procedure, transfer a volume of lysate to LSX strips, and test on VIDAS®. Post enrichment enumeration on MOX agar is determined with discrepant analysis on MOX agar after 48 hour enrichment in Fraser media.

Results: The Roka and VIDAS assays demonstrate 100% sensitivity and specificity, and the BAX assay demonstrates 94.4% sensitivity and 97.4% specificity. Additionally, Roka's method had a higher detection rate than BAX or VIDAS.

Significance: The Roka and VIDAS assays demonstrate improved sensitivity and specificity compared to BAX. However, Roka's method provides a higher detection rate of *Listeria* compared to VIDAS.

P1-45 Impact of Strain Variation on the Ability of Biosensor Technology to Detect *Salmonella enterica*

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Introduction: It is important to develop methods that can quickly and accurately detect the presence of bacteria in the food supply that cause disease. *Salmonella enterica* is often associated with contamination of food. Strains vary in their ability to cause illness and to spread. All methods that target cell surface structures for finding *Salmonella enterica* should be evaluated for their ability to detect different strains, because targets could be missing, masked or have alternative structures.

Purpose: This study tested the ability of biosensor technology to detect strains of *Salmonella enterica* known to vary in growth properties and cell surface structures. Some of the strains tested had mutations that altered the appearance of only one cell surface structure. Other strains had mutations in metabolic pathways, which could more globally affect the appearance of multiple structures on the outer membrane as well as growth properties.

Methods: CANARY® biosensors are B-cell lines that express monoclonal antibodies and the aequorin protein, so that luminescence is generated when the antibodies bind to their antigen. We tested PathSensor's CANARY® biosensor based technology for the ability to detect 18 different strains of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis). Nine other serotypes of *Salmonella enterica* were also tested. Strain variation within the set was maximized by deliberate mutation or by selection for the absence of cell surface molecules.

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Results: Of the 27 diverse *Salmonella* strains tested, one *S.* Enteritidis strain was found that was difficult to detect. It overexpressed a gene contributing to pili formation. In addition, *Salmonella enterica*that grew to high cell density impeded detection, because a high organic load interfered with signal development. Serial dilutions of organically loaded samples increased sensitivity for these strains. Strains varied in the lower limit of detection, which was between 30 and 300 CFU per sample aliquot.

Significance: Samples that contain *Salmonella enterica* can appear negative or weakly positive if cell concentrations are very high (> 10^7 CFU) or very low (< 10^2 CFU). In the case of highly concentrated samples, serial dilution increased signal. Only one strain was found that confounded detection at all cell concentrations and it is not found in nature. High organic load, originating from either a high target cell concentration or from an external mileau that dilutes target cells to less than a lower limit of detection, may interfere with detection by biosensor based technologies. CANARY® biosensor based technology accurately detected *Salmonella enterica* even when exceptional strain variation was present, but within parameters of optimal cell concentration.

P1-46 Investigating *Bacillus cereus* Behavior to Optimize Food Process and Surface Sanitation

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Introduction: *Bacillus cereus* group gathers closely related Gram-positive bacteria exhibiting highly divergent ecological and pathogenic properties. While *B. cereus* food poisoning outbreaks are mostly due to temperature abuse, the presence and survival of spores to food processes and cleaning procedures yield to their persistence in the industrial environment.

Purpose: The impact of environmental conditions on spore formation, resistance and inactivation upon various stresses was investigated to further optimize food formulation, industrial process and surface sanitation.

Methods: *B.weihenstephanensis* KBAB4, a psychrotrophic strain of *B. cereus* group was chosen as a model. Vegetative cells were grown in BHI broth and spore suspensions were produced in a specific synthetic medium. Survival kinetics upon various stress exposure were fitted using mathematical models to further simulate the impact of environmental conditions on spores and cells inactivation.

Results: Mathematical models describing heat resistance and spore formation as a function of the sporulation conditions were proposed. Higher spore formation and resistance were observed for conditions (temperature and pH) close to optimal growth conditions, yielding an easy screening of production steps at risk for spore contamination and germination. Moreover, acid inactivation of vegetative cells showed the presence of subpopulations with different resistance, more or less pronounced with mild stress pre-adaptation. Similarly, the impact of peracetic acid (temperature, concentration and contact time) was taken into account to further simulate spore inactivation by biocides.

Significance: While already available decision making tools enable the simulation of bacterial growth and heat destruction in food as a function of environmental conditions (pH, temperature, a_w), this study proposes several models to simulate bacterial inactivation upon exposure to acid or biocide. Even though further developments are needed to validate these results in food formulations and processes, these observations could be of importance for the food industry to refine safety procedures, as the presence of subgroups with different behaviors may greatly impact the resistance of the whole population.

P1-47 Pulsed Light Inactivation of *Salmonella* Enteritidis on Almond Surfaces

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Developing Scientist Competitor

Introduction: Almonds often are consumed raw and were the source of two outbreaks of *Salmonella* in 2001 and 2004. Several technologies like propylene oxide fumigation have been developed to reduce pathogens on almonds, but these have drawbacks such as hazardous chemical residues, deterioration of flavor and nutritional components, etc. Pulsed light, an emerging nonthermal technology, is proving to be effective against a myriad of microorganisms and food matrices without adversely affecting the food quality. Therefore, it shows great promise to be applied effectively for *Salmonella* inactivation on almond surfaces.

Purpose: To investigate the effect of pulsed light on the survival of a predetermined population of *Salmonella* Enteritidis(PT30), spot inoculated on the surface of almonds.

Methods: Spot-inoculated samples were exposed to pulsed light at a rate of 3 pulses/sec, for 10, 30 and 60 sec at a distance of 14.1, 16.6, and 19.2 cm from the central axis of the lamp. Samples were also intermittently treated for 60 sec with 10-sec treatment followed by a 3-min cooling period. The surviving population was enumerated.

Results: A maximum inactivation of 3.36 ± 0.19 log CFU/almond was obtained after a 30 sec exposure to pulsed light at a distance of 14.1 cm away from the lamp axis. Even a 10 sec treatment resulted in 2.88 ± 0.21 log CFU/almond reduction at 14.1 cm from the lamp axis. As expected, microbial inactivation decreased as the distance increased with reductions of 3.36 ± 0.19, 1.76 ± 0.09, 1.25 ± 0.04, and log CFU/almond were obtained at 14.1, 16.6 and 19.2 cm, respectively. Intermittent treatment of samples (10-sec treatment followed by 3-min cooling for 60 sec) resulted in up to 1.02-log CFU/almond increase in the microbial reduction depending upon the distance from the lamp housing.

Significance: Results clearly indicate that pulsed light is effective as a surface decontamination technology for inactivation of *Salmonella* on almond surfaces.

P1-48 Use of UV-C Light and Chemical Sanitizers to Inactivate Internalized *Salmonella* Typhimurium in Iceberg Lettuce

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Developing Scientist Competitor

Introduction: Washing with chemical sanitizers is the conventional procedure in fresh produce industry. However, it cannot effectively inactivate pathogens once they get internalized in plant tissues. Non-thermal treatments are warranted to kill the internalized pathogens, while maintaining the freshness and food quality of fresh produce.

Purpose: We investigated the practicality and efficiency of a combined treatment with UV-C radiation and chemical sanitizers for food industry to inactivate internalized human pathogens in fresh produce.

Methods: Iceberg lettuce was grown in the greenhouse for 4 weeks and then translocated to a growth chamber for *Salmonella* inoculation. All the lettuce was contaminated with green fluorescent protein-labeled *Salmonella* (10^8 CFU/ml) on the leaf surface and harvested in 2 days. Lettuce was rinsed with ethanol, $AgNO_3$ and DI water to remove the surface bacteria after collection. The internalized *Salmonella* was confirmed with plate

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count method and confocal microscope. For inactivating the internalized *Salmonella*, five groups of lettuce were treated for 10 min using different methods: UV-C (200 μW/cm^2; dose=1.2 kJ/m^2); chlorine (100 ppm); peracetic acid (PAA, 80 ppm); UV-C+chlorine; and UV-C+PAA. The reduction of internalized *Salmonella*in each treatment was determined with plate count method.

Results: *Salmonella* was observed in the interior region of the lettuce leaves. The internalization level was 2~3 log CFU/g of lettuce. When applying one single type of disinfectant, UV-C inactivated 98.9% (1.95 log) of the internalized*Salmonella*, which was higher than chlorine (89.3%, 0.97 log) and PPA (93.4%, 1.18 log). In contrast, the combined methods performed more effectively: UV-C+chlorine and UV-C+PAA reduced the internalized *Salmonella*by 99.8% (2.70 log) and 100% (2.87 log), respectively.

Significance: The internalized *Salmonella* in the lettuce was effectively inactivated applying the combined treatments with UV-C radiation and chemical sanitizers. This research provides a platform for future studies about inactivation of internalized pathogens with non-thermal processing and practical application of UVC irradiation in the fresh produce industry.

P1-49 Validation of Quaternary Ammonia and Hydrogen Peroxide Powder for Control of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Plants

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Introduction: *Listeria monocytogenes* is a pathogenic microorganism that is endemic in plant processing environments. Some best practices that are being utilized by ready-to-eat (RTE) processors seem to be working based on environmental monitoring data. Many times, however, these processes are not validated and questions remain regarding their true effectiveness.

Purpose: The objective of this study was to assess the effectiveness of two chemical interventions, quaternary ammonia (quat) and hydrogen peroxide (H_2O_2) powder, to control *Listeria monocytogenes* in RTE meat and poultry processing facilities.

Methods: To understand the effectiveness of dry, powdered quat and hydrogen peroxide against *L. monocytogenes* under commercial plant conditions; clean and soiled floor tiles at two moisture levels were tested. In addition, the effect of contamination level was investigated at two inoculation levels. A cold-adapted cocktail culture of seven strains of *L. monocytogenes,* including two quat-resistant strains was used for inoculation. A fully cooked turkey breast product was used to simulate RTE meat plant conditions.

Results: The survival of *L. monocytogenes* on the floor tiles was influenced by the presence organic components. Quat was not effective against *L. monocytogenes* on clean or soiled dry surfaces. Quat was also influenced by moisture and required moisture to become effective against *L. monocytogenes*. The efficacy of H_2O_2 and quat increased as moisture levels increased. H_2O_2 was effective against *L. monocytogenes* on clean or soiled dry surfaces. The efficacy of H_2O_2 was more significant in the presence of moisture.

Significance: Overall, H_2O_2 was more effective against *L. monocytogenes* under similar conditions compared to quat.

P1-50 Role of sigB and inlA Genes in Biofilm Formation and Antimicrobial Efficacy of Neutral Electrochemically Activated Water on *Listeria monocytogenes*

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Developing Scientist Competitor

Introduction: Neutral electrochemically activated water (NECAW) is a sanitizer for food and food processing equipment. *sigB* and *inlA* are stress response and virulence genes of *L. monocytogenes*, respectively, and it has been suggested that these genes play a role in biofilm formation and antimicrobial efficacy of NECAW treatment on *L. monocytogenes*.

Purpose: The objectives of this study were to determine the role of *sigB* and *inlA* gene expression levels in *L. monocytogenes* biofilm formation and their role on the antimicrobial efficacy of NECAW treatment.

Methods: Liquid cultures and biofilms grown on stainless steel coupon surface of four *L. monocytogenes* strains (wild type (WT) 10403S, isogenic Δ*inlA*, Δ*inlB*, and Δ*inlA*Δ*sigB*mutants) were treated with NECAW for 10 min. Sanitizing efficacy of NECAW was determined by counting the survivors after treatment using standard plate count. Gene expression levels were determined using qPCR.

Results: Isogenic *inlA* and *sigB* mutants were able to form biofilms. After NECAW treatment, both gene expressions increased for the WT. While *sigB* gene expression of Δ*inlA* strain increased comparable to the WT, *inlA* gene expression of Δ*sigB* strain did not significantly increase. Both genes were expressed more in biofilms than in liquid cultures. The level of *inlA* gene expression in WT increased by 4.28 and 5.51-fold with treatment with 4 mg/l NECAW for 10 min in liquid cultures and biofilms, respectively, while the corresponding values were 5.91 and 10.05-fold for *sigB* gene. Mutant strains were more sensitive to NECAW treatment than WT strain. For liquid culture, 10 mg/l NECAW for 10 min resulted in 0.79 and 1.17 more log CFU/ml reductions for Δ*inlA* and Δ*sigB*strains, respectively, than WT strain while there was no significant difference in biofilms under the same condition.

Significance: The *sigB* gene was more important than *inlA* for surviving NECAW treatment. Surviving *L. monocytogenes* cells post sublethal NECAW treatment might become resistant to further sanitizer treatment.

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P1-51 Evaluation of Biofilm Adaptability after the Use of Inadequate Disinfectant Solutions

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Introduction: Biofilm formation is a natural phenomenon occurring on industrial surfaces, most of the times undesirable because of the possible detachment of bacterial cells and further contamination of food products.

Purpose: To evaluate the disinfectant activity of inadequate concentrations of Benzalkonium chloride against bacterial biofilms.

Methods: In the context of ProSafeBeef EU project, mixed culture biofilms were formed on stainless steel surfaces in the combinations of *L. monocytogenes* (3 strains), *P. putida* (3 strains) and *L. sakei* (3 strains). The disinfection efficiency ofBenzalkonium chloride(20, 50, 100, and 200 ppm) was evaluated by detaching the remaining viable biofilm cells and enumerating by agar plating. The experiment lasted 10 days in total. Additionally, stainless steel surfaces exposed daily to disinfectant solutions and were tested for the recovering cells after their transfer to new medium.

Results: The population of *P. putida* was benefited by the presence of *L. monocytogenes,* and *L. sakei*, while the levels of 20, 50, and 100 ppm of the disinfectant were insufficient to suppress the biofilm populations and its efficiency was completely decreased as time elapsed. It is evident that

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after the 4th day of the experiment, the log reduction of microorganisms population after the disinfection ranged from 0 to 1 log CFU/g in all cases of mixed biofilms.

Significance: Such studies demonstrate the increased resistance of biofilms to common disinfectants, as well as the necessity of using the correct quantities for the complete elimination of the remaining biofilm cells.

P1-52 Evaluation of the Effectiveness of Five Sanitizers Using a Surface ATP Test System and Plate Counts

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Introduction: The bioluminescence measured by an ATP hygiene test system is dependent on the quantity of organic matter present on a surface. Lysed cells resulting from the action of sanitizers or the chemical composition of these compounds may influence the levels of Relative Light Units (RLU) measured by an ATP test system, giving false-positive RLU counts.

Purpose: The aim of this study was to evaluate the effectiveness of five commonly used sanitizers using 3M™ Clean-Trace™ Surface ATP Test System and plate counts.

Methods: Polyethylene plates (n=3) of 32 x 23 cm were divided in 8 squares (6 x 10cm^2) and were artificially contaminated with ~10^4 CFU/ml of *Listeria monocytogenes*, using a sterile sponge. Four squares of each plate were swabbed by 3M™ Swab-Sampler and four squares of each plate were swabbed with 3M Clean-Trace Surface ATP swabs, respectively. Samples were taken from control plates (untreated and uncontaminated) and from plates before and after the disinfection by sodium hypochlorite (1% and 2%), biguanide (0.6% and 1.2%), ethylic alcohol (70% and 96 °GL), quaternary ammonium (2% and 4%) and peracetic acid (1% and 2%). Data were statistically analyzed using Wilcoxon, Mann-Whitney and T-test, using Minitab® software.

Results: Results indicated that ~10^3 CFU/cm^2 of *Listeria monocytogenes*adhered to polyethylene plates, but no bacteria were found after disinfection with all sanitizers. The RLU counts of contaminated surfaces were ~3,000 RLU and were reduced to approximately 35-127 RLU after disinfection with all sanitizers. RLU counts verified after all sanitizers were considered statistically similar.

Significance: RLU counts were slightly higher than RLU counts verified on the controls, indicating the necessity of proper washing after the use of sanitizers and before RLU measures.

P1-53 Efficiency of Repeated Treatment with γ-irradiation to Eliminate Vegetative and Spore Forms of *Bacillus cereus* from Raw Rice

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Developing Scientist Competitor

Introduction: *Bacillus cereus* have been identified as causative agent in a number of emetic and diarrheic foodborne disease incidents.

Purpose: This study investigated the efficacy of repeated treatment with low-dose γ-irradiation to eliminate vegetative and spore form of *Bacillus cereus* ATCC 12480 from raw rice.

Methods: Rice samples artificially contaminated with spores and vegetative forms of *B. cereus*were treated with γ-irradiation at 0, 1.5, 3, 5, 7, 10, 15, 20, 25, and 30 kGy once, twice and thrice successive repeated treatment cycles, respectively, at 1.5, 3 and 5 kGy of γ-irradiation. Microbiological analysis for viable cells and spores count were performed after irradiation treatment; and field scanning electron microscopy was employed to determine the effect of irradiation on spore coat, size and morphology.

Results: Vegetative cells and spores of *B. cereus* in raw rice tolerated γ-irradiation up to 10 kGy and 20 kGy, respectively, at single treatment and were completely eliminated at 15 kGy and 25 kGy, respectively. Two and three cycles of irradiation treatment at 5 kGy eliminated all vegetative *B. cereus*. Repeated treatments were significantly more effective than single treatment at high dose ($P < 0.05$). There was no significant difference between two and three repeated treatment with γ-irradiation at the same dose ($P > 0.05$). Scanning electron microscopy images revealed that high doses of γ-irradiation slightly alter the morphology of spores by denaturing of exosporium at 20, 25 and 30 kGy. Also at 30 kGy the reduction in spore size and shrinking of spore coats was observed.

Significance: The efficacy of repeated treatment with low-dose γ-irradiation to eliminate *B. cereus* and the effect of γ-irradiation to viability, spore size and morphology was established. Elimination of foodborne pathogens from food is an ongoing challenge in which this study makes a scientific contribution.

P1-54 Isolation and Characterization of Bacteriophages for *Escherichia coli* O157:H7

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Introduction: *Escherichia coli* O157:H7 is an important human pathogen causing diarrhea, hemolytic-uremic syndrome, and hemorrhagic colitis. It has been detected in various food materials and products including cabbages, sprouts, ground beef, and vegetable juices. However, there are many difficulties in removing the pathogen.

Purpose: This study concerns the isolation of bacteriophage from cattle feces, characterization of stability, host range and morphology, and biocontrol for *E.coli* O157:H7 in vegetable products by using cocktail of the bacteriophages.

Methods: The stability test was performed under bacteriophage exposure to the diverse pH, alcohol, and heat conditions. The biocontrol experiment was conducted through comparing the number of viable *E.coli*O157:H7 in the vegetable juice and the surface of cabbage (2-3cm^2) by using cocktail of the bacteriophages.

Results: Four bacteriophages(ECP1, ECP2, ECP3, ECP4) were isolated from the cattle feces, and those showed high rate of infection to only 9 species of *E. coli* O157:H7 among 77 species of *E. coli*, and *Salmonella* spp. Morphological analysis by TEM indicated that all the bacteriophages belonged to Siphoviridae family. The burst sizes for every bacteriophages showed differences and those were about 40-100 PFU. all of bacteriophages were stable at pH2-pH11 and 70% alcohol concentration, however, they became disappeared at 70°C for 20 minutes. The bacteriophage cocktail, which is mixture of four bacteriophages, of isolation effectively prevented the growth of *E.coli*O157:H7 in the vegetable juice and surface of cabbage at 37°C in 5 hours.

Significance: Therefore, the host-specific bacteriophage to *E.coli* O157:H7 would help to control the pathogen in the vegetable products and probably in various foods.

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P1-55 Biofilm Removal from Stainless Steel Surfaces Using Abrasive Mechanical Disruption Combined with Low-volume Electrostatic Application of Sanitizer Spray

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Developing Scientist Competitor

Introduction: Bacterial biofilms on stainless steel food processing equipment compromise food safety and quality. Using a combination of two proven sanitation methods, abrasive mechanical disruption and charged electrostatic spraying of sanitizers, may yield an effective low-volume sanitation process to remove biofilms.

Purpose: As the first step in development of a process that would combine mechanical disruption and spray technology, this study evaluated using abrasive mechanical disruption (abrasive-particulate blasting) and air-assisted electrostatic-induction spray sanitizer application, in tandem, to remove biofilms from stainless steel surfaces.

Methods: An abrasive blasting chamber was fabricated so the abrasive blast stream was directed at stainless steel coupons containing *Listeria monocytogenes* biofilms. Coupons were positioned at a 60° angle horizontal to the spray and at distances of 8.9 and 24.1 cm from the nozzle. Each coupon was blasted with the abrasive (425-710 μm; 317 g/min) for 10 sec at 40 psi. Then levulinic acid and sodium dodecyl sulfate (SDS) sanitizer was applied so equal quantities of active ingredient were dispensed using either an air-assisted electrostatic nozzle with droplet charging ON (~ -7 mC/kg charge-to-mass) or with charging OFF or with a conventional hydraulic nozzle. For accurate spray application, nozzles were attached to a repeatable robotic arm. The methods were evaluated for the reduction in the population of colony forming units of *Listeria monocytogenes*previously inoculated onto the stainless steel surfaces.

Results: Abrasive mechanical disruption at a nozzle distance of 24.1 cm from the stainless steel surface combined with air-assisted charged spray worked best and reduced the number of biofilm cells by 5.28 logs CFU when using a sanitizer comprised of 6% levulinic acid and 0.6% SDS.

Significance: Bacterial biofilms present significant obstacles in food processing environments. Development of a process that can deliver abrasive mechanical disruption and low-volume electrostatic application of sanitizer spray may provide an effective means for biofilm control.

P1-56 Use of Bacteriophage Cocktail for Biocontrol and Biofilm Removal of *Bacillus cereus*

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Introduction: *Bacillus cereus* is a spore-forming foodborne pathogen which can cause emetic or diarrheal syndromes. Because it may form biofilms under various conditions, it can be difficult to control in foods. Bacteriophages are a potential means of controlling *B. cereus.*

Purpose: The aims of this study were to isolate bacteriophages of *B. cereus*from various environments and to identify their characteristics.

Methods: After isolating the bacteriophages by the plaque assay, they were characterized by their host spectrum, one-step growth curve, stability, and morphological characteristics using transmission electron microscopy (TEM). A bacteriophage cocktail was used to study bio-control of *B. cereus* in food and biofilm removal on glass wool.

Results: Among the nineteen bacteriophages that were isolated, four showed a broad host spectrum on various strains of *B. cereus*. TEM analysis showed that bacteriophages BCP3 and BCP11 belonged to the Siphoviridae family, and BCP9 and BCP18 belonged to the Myoviridae family. Their burst sizes were approximately 130 - 160 PFU. They generally showed stability to low pH, and exposure to 50% and 70% (v/v) ethanol for 30 min caused a 5- and 7-log PFU/ml decrease, respectively. They showed instability to 70°C after 40 minutes. A cocktail of the four bacteriophages caused a 5-log CFU reduction of *B. cereus* in radish sprouts after 3 hours. After a 4-h treatment with the bacteriophage cocktail, biofilm-forming *B. cereus* was not detected on the glass wool.

Significance: Bacteriophages have the potential to control *B. cereus* in foods or in biofilms.

P1-57 Superficial Contamination of Conveyor Belts in Chicken Cutting Area: Effect of Cleaning by Water Spray at 45°C

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Introduction: Conveyor belts, which are continuous cutting surfaces, are regularly used in meat-cutting areas, specifically in poultry slaughterhouses. Operational cleaning procedures for these conveyor belts are based on spraying warm water, generally at 45 °C and under pressure. However, water may spread contamination to the products, and not effectively reduce organic material on surfaces.

Purpose: The objective of the present study was to compare microbial counts in conveyor belts submitted or not to continuous cleaning by water spray at 45°C.

Methods: A total of 216 superficial samples were collected at three different times (T1, T2 and T3), in both conveyor belts (with or without continuous cleaning with water at 45°C). The following times were used for sampling: T1 - soon after the preoperational cleaning procedure; T2 - at the turn of the work shift; T3 - soon after the second work shift; completing the two work shifts of conveyor belt use. Mesophilic aerobes and Enterobacteriaceae counts were performed.

Results: For Enterobacteriaceae, no significant differences ($P > 0.05$) were observed between the conveyor belts, independent of the time of sampling or cleaning process. No significant differences ($P > 0.05$) were observed between the counts of mesophilic obtained in distinct times of sampling in the conveyor belt not submitted to continuous cleaning with water at 45°C. Comparing similar periods of sampling, no significant differences ($P > 0.05$) were observed between the mesophilic counts obtained from conveyor belt submitted or not to continuous cleaning with water at 45°C. The continuous cleaning with water did not promote a significant reduction of mesophilic and Enterobacteriaceae counts, suggesting the possibility of discarding this procedure in chicken processing in order to reduce the emission of effluents in the environment.

Significance: Based on these evidences, alternative cleaning processes must be proposed and evaluated to provide proper microbial load reduction in poultry processing facilities equipments.

P1-58 Efficacy of an Isopropyl Alcohol Quaternary Ammonium Formula and Carbon Dioxide Sanitizer System for Reducing *Salmonella* on Food Contact Surfaces

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Introduction: Effective cleaning and sanitation operations directly impact the production of safe foods. Dry processing environments are particularly challenging to clean and sanitize because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella*.

Purpose: The objective of this study was to determine the efficacy of a nonflammable isopropyl alcohol quaternary ammonium formula (IPAQUAT) and carbon dioxide sanitizer system for reducing *Salmonella* spp. on food contact surfaces.

Methods: Coupons of stainless steel and conveyor belting material typically used in dry processing environments were spot-inoculated in the center of 5 x 5 cm coupons with approximately 10.0 logs CFU/ml of a six-strain cocktail of*Salmonella* spp. and subjected to a treatment of IPAQUAT formula. After treatments of 30 s, 1 or 5 min, wet coupons were swabbed for enumeration and recovery. Duplicate inoculated surfaces were soiled with a breadcrumb flour mixture and allowed to sit on the lab bench for a minimum of 16 h before IPAQUAT treatment of 30 s, 1 or 5 min and then swabbed for recovery. For clean and soiled surfaces, serial dilutions were prepared and pour plated using tryptic soy agar plates for recovery and Hektoen enteric agar for *Salmonella* confirmation.

Results: While approximately 7 logs CFU/coupon were recovered before treatment, an average of 1.2 logs CFU/coupon , 0.8 logs/coupon, 0.7 logs/coupon were recovered after 30 s, 1 or 5 min treatments, respectively, for both clean and soiled surfaces. Treatment of IPAQUAT for 30 s resulted in 5.8 log CFU/coupon reductions whereas, greater than 6.2 log CFU/coupon reductions were observed for treatment times of 1 and 5 min.

Significance: Therefore, IPAQUAT system reduced over 6 logs CFU/coupon of *Salmonella* spp. when applied for 1 min or more and would be an effective sanitation system for dry-processing environments.

P1-59 Presence of Norwalk Virus RNA on the Hands of Infected Individuals

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Introduction: Human noroviruses (NoVs) are the leading cause of foodborne gastroenteritis outbreaks in the world. Outbreak investigations have shown that food handlers play an important role in NoV person-to-person transmission, however, there is no experimental evidence demonstrating the presence of NoV on the hands of NoV-infected individuals.

Purpose: To evaluate the presence of Norwalk virus (NV) RNA on the hands of volunteers orally challenged with NV-seeded oysters.

Methods: This study was incorporated into a clinical trial that evaluated NV inactivation in oysters by high hydrostatic pressure processing (HPP). Forty-four adults were orally challenged with NV in artificially seeded oysters with or without HPP treatment, and 13 subjects became infected. During the acute phase of follow-up, 159 handrinse samples were collected from 6 infected and 6 uninfected subjects on days 3-5 post-NV challenge. Viruses were precipitated with polyethylene glycol followed by viral RNA extraction with the EasyMAG method. NV RNA was quantified using a NV-specific real-time RT-PCR assay with an internal PCR amplification control and RNA standards.

Results: A total of 40.8% (29/71) of the handrinse samples collected from 6 infected volunteers screened presumptively positive for NV, with an average of 3.56-log genomic equivalent copies (GEC) of NV detected per positive hand rinse sample. The NV detection rates and geometric mean titers showed no significant differences ($P > 0.05$) between samples collected immediately after bathroom use and during routine vital sign measurements. In addition, NV titers in handrinse samples and fecal samples collected on the same day showed no clear correlation. Efforts are currently underway to confirm presumptively positive samples.

Significance: These findings provide further evidence that NoV contamination on human hands is important, but does challenge current paradigms that huge quantities are omnipresent on the hands of those infected. Developing effective hand hygiene strategies to prevent NoV transmission and infection is critical in work places. This research was approved by an Institutional Review Board.

P1-60 Microbiological Evaluation of Carcass Transport Vehicles and a Survey on the Sanitary Performance at the Meat Cutting Plants in Korea

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Introduction: Meats are generally transported as carcasses or packaged form in boxes in Korea. Carcasses can be exposed to biological, chemical and physical hazards during transportation, and therefore the hygienic management of meat before reaching the processing stage is of utmost importance for the processing quality of meat. For this, the stage of meat transportation is applied mandatorily by Sanitary Standard Operation Procedure (SSOP) and voluntarily by HACCP in Korea.

Purpose: The purpose of this study was to evaluate the microbiological contamination levels of carcasses as well as vehicle environment applied by only SSOP or HACCP.

Methods: The investigation was carried out targeting carcass transportation vehicles immediately after their arrivals at meat cutting plants. The subjects were made up of totally 3 groups, HACCP applied ones (A) and non-HACCP applied ones belonging to cutting plant (B) and rent (C). Microbiological contamination levels were evaluated using aerobic plate count (APC), coliforms and pathogenic bacteria, such as *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*. The samples were collected from floor, wall and utensils of cargo box, carcasses and employees' gloves and apron. Sanitary performance of employees was conducted using the questionnaire.

Results: The number of APC of cargo box floor was significantly higher ($P < 0.05$) in the non-HACCP applied vehicles, with the mean value of 3.9 (B) and 4.2 log CFU/cm^2 (C), respectively compared with 3.2 log CFU/cm^2 in the HACCP applied ones (A). Coliforms were also detected with the higher numbers of 1.2 (B) and 1.4 log CFU/cm^2 (C), respectively, for cargo box floor, compared to 0.6 log CFU/cm^2 in the Group A. *S. aureus* and L. *monocytogenes*were only detected in the non-HACCP applied groups (B and C) whereas they were not detected in the HACCP applied group (A). In the sanitary performance, the total mean scores of sanitation performance, cargo box management were the highest score in the employee of HACCP applied vehicle.

Significance: With the results, the application of HACCP system to meat transportation stage is regarded to improve the hygienic management conditions and therefore the enlargement of HACCP application in this area will be needed in Korea.

P1-61 Evaluation of Alcohol Wipes and Microfiber Cloths for Cleaning Dairy-Contact Stainless Steel Surfaces

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Introduction: Wet-cleaning practices in dairy processing may not be the optimum solution to controlling pathogenic bacteria in all dairy manufacturing plants, for example during powder manufacture. Alternate strategies to clean dry areas of dairy manufacturing are needed.

Purpose: The aim of this study was to evaluate the use of alternate cleaning methods, such as alcohol-impregnated wipes and microfiber cloths, for dairy-contact stainless steel surfaces.

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Methods: Stainless steel surfaces (30 cm X 30 cm) were inoculated with overnight cultures of *Staphylococcus aureus* ATCC 25923 either resuspended in reconstituted skim milk medium (RSM), or freeze-dried and dry-blended with milk powder (worst-case scenario – 10^6cells). The inocula were left on the surfaces for either 1 or 18 h before cleaning. Surfaces were wiped using various microfiber cloths or sponges (n=4) or antimicrobial-impregnated wipes (n=3), and comparisons made to a commercial antimicrobial spray and an untreated control. Surviving cells were enumerated using standard microbiological procedures on three separate occasions and significant differences (95%) between the counts determined.

Results: Overall, the alcohol-impregnated wipes performed best throughout this study (*ca*. 90% reduction), while the microfiber cloths and sponges only showed some efficacy in removal of *S. aureus* cells in milk powder residue (*ca*. 80% reduction). Ethanol-, isopropanol- and mixed antimicrobial–impregnated wipes significantly ($P < 0.05$) reduced populations of *S. aureus* in liquid milk residue that had been dried onto stainless steel surfaces for both 1 and 18 h. By comparison, no significant reductions ($P > 0.05$) in the populations of *S. aureus*in milk powder residues on stainless steel surfaces were obtained with any cloth or wipe tested.

Significance: Results showed that traditional cleaning of dairy processing surfaces remains important for keeping pathogen numbers low (under control). Alcohol-impregnated wipes may be suitable alternate strategies to aid in such cleaning regimes in dry areas of dairy manufacturing plants, but only if attached population numbers are low. None of the wipes tested in this study resulted in more than 93% reduction in pathogen numbers.

P1-62 Cleaning and Sanitation of *Salmonella*-contaminated Peanut Butter Processing Equipment

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Introduction: Contamination of peanut butter and nut butter products by pathogenic *Salmonella* serovars have led to an increasing number of product recalls and foodborne outbreaks. Post-processing contamination of peanut butter poses a significant health risk to consumers as *Salmonella* can remain viable throughout the shelf-life of the product. Effective cleaning and sanitation of nut butter lines are essential for preventing cross-contamination of microbial hazards such as *Salmonella.*

Purpose: The objective of this study was to evaluate the efficacy of commonly used commercial cleaning methods, hot oil and isopropanol, on *Salmonella*survival and/or removal from pilot-scale peanut butter processing equipment.

Methods: Peanut butter inoculated with a cocktail of four *Salmonella* serovars (inoculation level ~7 log CFU/g) was used to contaminate peanut butter processing equipment (capacity ~75 l). The system was drained of peanut butter, and treated with hot oil (93°C) for 2 h and then 60% isopropanol for 1 h (via continuous recirculation). Microbial analysis of environmental (swabs of 8 different locations in the processing line) and product (peanut butter and oil) samples obtained during the cleaning procedures was conducted using trypticase soy agar with yeast extract (TSAYE) and xylose lysine deoxycholate (XLD) agar.

Results: Samples of oil obtained after the 2 h cleaning treatment contained ~3.2 log CFU/g on both TSAYE and XLD, indicating the hot oil treatment was not sufficient to inactivate the contamination in the processing line. Following hot oil circulation, environmental sampling of various locations in the processing line found 2.5-7.0 log CFU/cm^2 remaining on processing equipment surfaces as measured on TSAYE and XLD. After the 60% isopropanol sanitation treatment, no *Salmonella*was detected in environmental samples cultured on XLD (detection limit = 1.0 log CFU/cm^2).

Significance: These data suggest that a two-step process consisting of a hot oil cleaning step followed by a 60% isopropanol sanitization treatment may eliminate pathogenic *Salmonella* from a contaminated peanut butter processing line.

P1-63 Survival of *Listeria monocytogenes* on Stainless Steel Exposed to Dry Heat

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Introduction: *Listeria monocytogenes*, the causative agent of listeriosis and a major concern for the food industry, is widespread in the environment and may be introduced into food processing facilities resulting in contaminated ready-to-eat (RTE) foods. While most RTE dry foods are generally considered not to support the growth of *L. monocytogenes* they may, in some cases, be used as ingredients for RTE foods that do support *Listeria* growth. Many dry food manufacturing facilities maintain a dry environment for lengthy periods followed by a wet cleaning and sanitation cycle. Very limited data is available regarding survival of *Listeria*on non-porous dry surfaces exposed to dry and moist heat.

Purpose: The purpose of this study was to investigate the ability of *L. monocytogenes* to survive on stainless steel in dry and moist heat conditions.

Methods: *L. monocytogenes*ATCC 49594 was inoculated onto stainless steel carriers and exposed to several different dry and moist heat conditions. After the indicated exposure times the organisms were recovered and log reductions were calculated.

Results: A 2-log reduction was achieved after 4 h at 90°C dry heat while over a 3-log reduction was achieved after 1 h at 100°C dry heat. When coupons were soiled with 10% whole milk or 5% lactose and exposed to 100°C dry heat, a > 3-log reduction was achieved after 30 min. When a 5% glucose solution was used the same reduction was achieved after 15 min. In the presence of steam (80-90°C) a > 3 log-reduction was observed after 5 min with or without the whole milk or carbohydrate soils.

Significance: This study indicates dry heat can eliminate *Listeria monocytogenes* from non-porous surfaces, such as stainless steel, but will require extended time periods and/or high temperatures.

P1-64 Cross-contamination Risks in Reusable Grocery Shopping Bags

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Introduction: Reusable polypropylene-grocery shopping bags have increased in popularity as a method to reduce environmental impact of plastic one-use bags. In 2010, researchers found eight percent of reusable bags tested were positive for *Escherichia coli* (*E. coli*).

Purpose: It is important to better understand the potential reusable bags pose in cross-contamination of foodborne pathogens. This study considered the potential of pathogen transfer from contaminated leafy greens to a reusable bag and survival on a reusable bag.

Methods: Leafy greens were inoculated with 10^5 CFU/ml tetracycline and chloramphenicol resistant *E. coli* O157:H7. Inoculated and non-inoculated leafy greens were placed in reusable bags (24 bags per treatment) and 30-minute transport was simulated using a large sample mixer. The microbial load of the reusable bags was measured one-hour after simulation and all bags were stored at 21°C. Three bags per treatment were tested on Day 1, 3, 5, 7, 9, 11, and 14. Bags were sampled in five locations (10 x 10 cm), one on the bottom and four at varying levels on the side-panels of the bag, using wet swabs. Reusable bags were measured for aerobic plate count, yeast and mold, coliform, and *E. coli* O157:H7.

Results: One-hour after transport simulation and on Day 1, 10^3 CFU/ml tetracycline and chloramphenicol resistant *E. coli* O157:H7 was recovered. Microbial loads from Day 3 to Day 14, 10^1 CFU/ml *E. coli* O157:H7 was consistently recovered. Counts were similar regardless of

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sampling location on the bag. Non-pathogenic microorganisms were recovered at low concentrations.

Significance: It is important to consider potential pathogens available for transfer to reusable bags and further food products. The data generated helps with the development of a better model for assessing microbial movement within reusable bags. The results of this study convey the potential for survival and cross-contamination of foodborne pathogens on reusable bags.

P1-65 Effect of Detergent and Food Residues on the Survival of *Listeria monocytogenes* and *Salmonella* spp. in Sponges

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Introduction: Investigating the behavior of pathogens in microenvironments formed by food residues in sponges with or without detergent would help us understand and improve the household sanitation.

Purpose: To evaluate the behavior of *Listeria monocytogenes* and *Salmonella* spp. strains in sponges containing homogenates of chicken, mayonnaise and yogurt with and without detergent, after consecutive treatments with detergent or plain water simulating the household use.

Methods: Three strains of *Listeria monocytogenes* and *Salmonella* Typhimurium or Enteritidis were inoculated (10^4 - 10^5 CFU/ml) in homogenate of yogurt (5% w/v), mayonnaise (5% w/v) or chicken (1% w/v) of pH 4.5, 4.5 and 6.5, respectively. Then, aliquots (3 ml) of a homogenate were poured in commercial sponges and incubated at 20°C for up to 7 days. Inoculated sponges were treated with a commercial detergent (1% w/v) which proved to be the most effective among 4 detergents with different composition. Sponges were dipped and squeezed in tap water containing detergent (1% w/v) resulting in the sponge holding 10 ml of liquid. Successive treatments of sponges with water or detergent took place on days 1, 4 and 7 at 20°C.

Results: Both pathogens increased to 2.3 - 2.5 log CFU/g during incubation of sponges in the absence of detergent. After the first treatment with the detergent, *L. monocytogenes* reduced by 1.7–2.1 log CFU/g and *Salmonella* by 0.7-1.4 logCFU/g. Consecutive treatments with detergent caused further reductions (0.3-1.3 log CFU/g) within 7 days to those observed on the first day. In contrast, repeated treatments of sponges with water resulted in growth of up to 2.1 logs CFU/g of both microorganisms. Repeated treatments of sponges with detergents delivered 2.3 log CFU/g higher reductions than a single treatment on day 1, followed by storage without further treatments.

Significance: The survival and growth of pathogens in sponges suggest that sponges are critical vehicles of bacterial contamination in household environments.

P1-66 Norovirus Inactivation Using Chlorine Dioxide Gas on Stainless Steel Coupons

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Developing Scientist Competitor

Introduction: Acute gastroenteritis caused by human norovirus is becoming a significant public health issue. Fresh produce and seafood are examples of high risks foods associated with norovirus outbreaks. Chlorine disinfectant, at the dosage recommended by the Food and Drug Administration, is not effective in norovirus inactivation. Previous studies have shown that chlorine dioxide gas is an effective antimicrobial agent for different pathogenic bacteria on fresh produce.

Purpose: The objective of this study was to determine the effectiveness of chlorine dioxide gas to inactivate norovirus on stainless steel coupons that could mimic food contact surfaces in the food industry.

Methods: The cultivable murine norovirus (MNV-1) was used as a surrogate for human norovirus and was inoculated on stainless steel coupons at the concentration of 10^8PFU/ml. The samples were treated with chlorine dioxide gas at 1 mg/l and 8 mg/l for 1, 5, and 15 min at 25°C. Viral plaque assays were used for quantification of recovered viral particles.

Results: At a concentration of 1 mg/l, there was approximately a 1.5-log reduction in MNV-1 particles after treating the stainless steel coupons for 15 min. At the higher concentration (8 mg/l), no plaques could be found after treatment of 1, 5, or 15 min which resulted in a > 5-log reduction in MNV-1 particles.

Significance: Chlorine dioxide gas at 8 mg/l effectively inactivates human norovirus surrogate. This treatment method yields promising results for potential use on food contact surfaces and possible for food surfaces. More research should be completed to better understand inactivation kinetics for norovirus in high risk foods (such as fresh produce and seafood) in an effort to reduce the incidence of norovirus-related outbreaks.

P1-67 Inactivation of Norovirus Surrogates by UV Irradiation and Chlorine Disinfection on Stainless Steel Surfaces and Development of Predictive Reduction Models

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Developing Scientist Competitor

Introduction: Norovirus, as the most serious foodborne virus, is a cause of epidemic gastroenteritis worldwide. Combined treatment of UV irradiation and chlorine are commonly used in the food industry to reduce norovirus on surfaces for food preparation and manufacturing.

Purpose: This study was conducted to identify the maximum synergistic effect points of UV irradiation and chlorine treatment for inactivating feline calicivirus strain-9 (FCV-9) and murine norovirus type 1 (MNV-1) on stainless steel surfaces and to develop predictive reduction models using response surface methodology (RSM).

Methods: As surrogate models of norovirus, FCV-9 and MNV-1 were used in this study. The reduction levels of FCV-9 and MNV-1 on stainless steel surfaces were investigated by UV irradiation (0- 120m W ·s/μ²) and chlorine concentrations (0-5,000 ppm). The Polynomial models were developed for predicting reduction models of FCV and MNV by UV irradiation and chlorine disinfection.

Results: The reduction levels of FCV were steadily increased in the 0-80 mW ·s/μ² of UV irradiation at the higher chlorine concentration. However, chlorine disinfection did not affect reduction of FCV in the 80-100 mW ·s/μ² of UV irradiation. The reduction levels of MNV at the treatment of 3,000-4,000 ppm chlorine were much more significantly affected at 60-120 mW ·s/μ² of UV irradiation than at 0-60 mW ·s/μ² of UV irradiation. The polynomial equations predicting the inactivation of FCV and MNV were as follows: FCV (log $TCID_{50}$/coupon) = $1.294+0.007 x_1^a + 0.0003 x_2^b + 0.0002 x_1^2$ (x_1: UV and x_2: chlorine); and MNV (log $TCID_{50}$/coupon) = $1.638-0.026 x_1 + 0.0008 x_2 + 0.0003 x_1^2$ (x_1: UV and x_2: chlorine). The predictive reduction models by RSM were fit well ($R^2 = 0.9939$ and 0.9332) and expressed as adequate models by Prob > F-value ($P < 0.0001$).

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Significance: Combined treatment of UV irradiation and chlorine disinfection provides effective reduction of norovirus on stainless steel surfaces and the predictive reduction models of FCV and MNV could be used in food manufacturing facilities.

P1-68 Sporeforming Bacteria: Biodiversity and Prevalence in Food Industries

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Introduction: Aerobic and anaerobic Gram-positive sporeformers exhibit a wide range of phenotypic and genotypic characteristics. These organisms are ubiquitous in the environment and have the ability to form endospores which enable them to survive heat treatments and sanitation commonly used in food process. The presence of spores in food may be associated with characteristic spoilage activity when bacterial germination and outgrowth is possible. Indeed during the last decade, the achievement of commercial sterility requirements has been hampered by the presence of psychrotrophic or highly heat-resistant sporulated bacteria in pasteurized and sterilized products.

Purpose: The aim of this study was to evaluated biodiversity and prevalence of various sporeforming bacteria commonly found in raw material, ingredient, dehydrated products and ready-to-eat food, with and without visible trace of spoilage.

Methods: Spore counts, 16SrDNA sequencing isolate identification and molecular detection of targeted sporeformers were performed on raw material, ingredient, ready-to-eat and after surface samplings along industrial production lines. Further phenotypic characterizations were also done on some isolates, such as spoilage properties, heat resistance and minimal growth conditions.

Results: A wide diversity of sporeformers was recovered from the studied samples. Spore contamination was mainly associated to raw material, dehydrated ingredients, concentrates and environment. Even though multiple contamination sources were identified, no spore formation area nor cross contamination between surface/products was highlighted. For studied industrial sites, low contamination could be associated to good hygiene conditions and food product storage in conditions which do not allow bacterial growth.

Significance: Sporeformer diversity and prevalence from raw material to end product strongly suggest the emergence of thermophilic strains. This phenomenon might be due to the use of food ingredients and modern processing technologies that select sporeformer contaminants which survive sub-lethal stress conditions, thus leading to the persistence of specific strains in food industries. When spore contamination is detected along a production, screening or pre-treating raw food and ingredients would be a wiser option than always increasing heat treatments or processing.

P1-69 Chlorine Treatment and Lactic Acid Bacteria Application for Reduction of Spoilage Microorganisms from Clover Seeds and Sprouts

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Introduction: Sprouted seeds have become a major food commodity due to their complete nutritional profile. However, consumption of raw sprouts poses significant health risks on account of the warm, humid growing conditions that foster the growth of foodborne pathogens. Similarly, contaminated water and seeds, inadequate ventilation and excessive heat can promote the growth of food spoilage microorganisms. Approaches aimed at antagonizing the growth of these bacteria have included application of chemical sanitizers to the seed surface and inoculation with probiotic bacteria.

Purpose: To evaluate the effectiveness of chlorine treatment and the antagonistic effect of lactic acid bacteria (LAB) against spoilage bacteria in clover seeds and sprouts germinated under sterile growing conditions.

Methods: Crimson clover seeds (Oregon Grown, OR) were disinfected with Clorox at 5,000 and 20,000 ppm at 35°C for 15 min. After thorough rinsing, seeds were soaked in an IEH-screened LAB cocktail at 10^6 CFU/ml concentration for 5 min or LAB was inoculated directly into the agar substrate. Controls consisted of seeds that were unbleached, and/or uninoculated. Seeds were stored at 4°C overnight and then germinated in a sprouter drum for 24 hr. Percent germination was ascertained and sprouts were then planted in sterilized clam shells containing 0.5% agar inoculated with 10^6CFU/ml LAB. Aerobic plate counts (APC) were assessed before and after each treatment. Fully matured sprouts were then stored at 4°C to enable monitoring of shelf life.

Results: Treatment with Clorox at 20,000 ppm attenuated APC on clover seeds by 2 logs relative to untreated controls with no significant effect on seed germination and/or sprout establishment. Soaking seeds with LAB prior to germination, as well as adding into substrate prior to sprouting in clam shells likewise imparted a reduction of APC by 2 logs. *Pseudomonadaceae*, *Enterobacteriaceae*, and *Bacillaceae*were the major spoilage bacteria identified. Application of these techniques extended shelf life under 4°C refrigeration by approximately one week.

Significance: The presence of spoilage pathogens in raw sprouts generally indicates shorter shelf life. Thus, proper seed sanitization methods would limit potential financial losses and diminish the risk of foodborne illnesses associated with sprout consumption.

P1-70 The Metagenomic in the Service of the Food Microbiology

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Introduction: Food products represent great biotopes for bacteria. The optimization of foodstuffs conservation, mattering so economically as from the point of view of the public health, pass by a better understanding of those biotopes and their spoilage. Microbiologists had already tried to resolve this problem throughout several approaches. Studies based on classical microbiology cultures were completed by strategies centered on approaches independent from the microbiological culture.

Purpose: The current techniques of new generation sequencing give a new dimension to the microbial ecology through the metagenomic analysis of individuals' large number within a mixed microbial population. Our aim is to demonstrate that this methodology can be successfully applied to the study of foodstuffs microbial flora and can be adapted to the specific requirements of food microbiology.

Methods: This study was carried out on pork's minced meat and white sausage, with shelf life tests in various conditions of preservation (temperature and packaging). The rDNA 16S was extracted from the original products and samples in the best-before date and, after standardization, hypervariable regions V5 and were sequenced.

Results: A total about 130,000 sequences were obtained and a metagenomic analysis succeeded in the taxonomic classification to the genius level for 80% of this population. The subsequent analysis of microbial populations shows that the majority microbial populations at the expiration date are the same ones which are generally observed during microbiological analysis of these meat products. However, the population subdominants and especially several populations of not cultivable germs were able to be identified. These groups of bacteria, more difficult to obtain by the other methods, must be studied because they participate in the spoilage process of food products.

Significance: The sensibility of this technology makes possible the analysis of foodstuffs presenting a very low microbial rate and thus allows the identification of the microbial contaminants before they grow to levels detected by cultural methods.

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P1-71 Freshness and Quality Change in Tofu Measured by Glucose Level

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Introduction: Tofu is a healthy, popular and traditional soy food in Asian diets, since it is rich in protein, calcium, B-vitamins, iron and magnesium. However, it is very sensitive to microbial growth even under refrigeration because of its relatively high moisture content and pH. In quality deterioration of tofu, the level of glucose could be one of the excellent indicators.

Purpose: The purpose of this study was to evaluate the freshness and quality change of tofu during storage under refrigeration after opening it. The relationship between freshness of tofu and glucose concentration was determined.

Methods: The degree of freshness was measured by glucose concentration, total bacterial counts, surface pH and sensory evaluation. To estimate glucose concentration in tofu, glucose oxidation assay was carried out. APHA (American Public Health Association) method was used for the examination of total bacterial counts.

Results: After storage of 5 days at 4°C Tofu had a change in decrease of glucose concentration by 20.3%, and its pH and total bacterial counts reached 6.1 and 2.80 log CFU/g, respectively. In the equation of relationship between glucose concentration and microbial growth is (where y = glucose concentration and x = total bacterial counts). Glucose concentration and microbial growth are in inverse proportion to each other.

Significance: Based on this study, the freshness of tofu could be evaluated by measuring glucose concentration, establishing a new freshness indicator for tofu.

P1-72 Genotypic Characterization of *Brochothrix thermosphacta* Strains that Developed during Storage of Minced Pork under Aerobic or Modified Atmosphere Packaging Conditions

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Introduction: *Brochothrix thermosphacta* is among the major member of the meat microbial asscociation that can cause spoilage in chilled meat either pork or beef stored in modified atmosphere packages.

Purpose: The aim of the present study was to determine the genotypic diversity of *Br. thermosphacta* at strain level that developed during storage of minced pork under different conditions.

Methods: Minced pork was stored at 0, 5, 10 and 15°C, aerobically and under modified atmospheres packaging (MAP). Thus, a total of 294 *Brochothrix* isolates were recovered from initial, middle and final time points of storage of minced pork samples. The *Brochothrix* heterogeneity at strain level was performed with pulsed field gel electrophoresis (PFGE).

Results: This organism dominated at all temperatures under MAP conditions. Macrorestriction analysis of DNA of 294 *Brochothrix* isolates revealed a high diversity depending mostly on temperature, while the obtain fingerprints were divided in 4 different groups. More specifically, the first group consisted of the isolates from 0°C of air package and isolates from fresh meat from the batch used for packaging under aerobic conditions, the second group consisted of 4 strains, 3 isolates of final stage of 10°C, and one isolate of fresh meat. The third group consisted of isolates recovered from middle and final time point of MAP samples stored at 0°C, one isolate of air packaging at 0°C and three isolates of fresh meat from the batch used for MAP. Finally, the last group consisted of the remaining strains. Representative isolates from each group were subjected to 16S rRNA gene sequencing and assigned to *Br.thermosphacta*.

Significance: These observations are of great importance and are fundamental in widening the knowledge of meat microbial ecology and specifically spoilage-related bacteria and consequently understanding the meat spoilage process.

P1-73 Conventional Methodologies vs. Metabolomics for the Quantification of Spoilage of Beef Filets and Minced Beef

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Introduction: Meat industry needs rapid analytical methods or tools for the quantification of meat spoilage and for the estimation of the product's shelf life.

Purpose: To evaluate metabolomics as a potential tool to quantify meat spoilage.

Methods: In the context of SYMBIOSIS - EU project, ground meat and beef filets were stored aerobically, under modified atmosphere packaging (MAP) and under MAP with the presence of the volatile compounds of oregano essential oil (MAP/OEO) at 0, 5, 10 and 15°C. The microbial association, i.e., total viable counts, pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria, *Enterobacteriaceae*, yeasts/molds, was assessed in parallel with sensory analysis, pH measurements and the evolution of the metabolic products, as well as the compounds occurring in the meat substrate using HS/SPME-GC/MS and HPLC.

Results: The data collected by HPLC and GC/MS were correlated with microbial counts and sensory scores to estimate the shelf life of the beef, aiming mainly at the early detection of spoilage. Both parameters, i.e., temperature and packaging, were found to have a great impact on the evolution of end-products during storage that resulted in distinct profiles in both minced and beef filets. Correlation of the metabolic profile, monitored with these instruments and developed during beef storage, with the sensory discrimination of the samples, was performed with principal components analysis (PCA) and factorial discriminant analysis (FDA), whilst quantitative predictions of the different microbial groups were performed with partial least squares-regression (PLS-R). The performance of these models for the different microbial groups was 75.34 to 91.78% within the ± 20 % relative error.

Significance: Overall, it was shown that metabolic profiling derived from HS/SPME-GC/MS and HPLC analysis of meat combined with advanced computational analysis may be considered as a potential method to predict the spoilage of a meat sample regardless of packaging type and storage temperature.

P1-74 Bacterial Growth and Histamine Production in Tuna Salad Preparations

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Introduction: Histamine (Hst) poisoning is associated with consumption of scombroid fish species such as tuna. Contamination of commercial tuna salad with Hst-producing bacteria (HPB) from raw tuna, processing equipment or added ingredients, combined with temperature abuse during processing/storage, can present a food safety hazard.

Purpose: The objectives of this study were to (1) isolate HPB from onions and celery to determine their role in contamination of commercially-produced tuna salad and (2) evaluate Hst production by HPB during storage of tuna salad containing added ingredients.

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Methods: Bacteria were isolated from onion and celery using MRS or TSB+1% histidine broths and characterized by real-time PCR, API 20E, 16S sequencing, and/or Hst production. Twenty-five g of tuna salad (3:1 tuna:mayonnaise) and tuna salad with added ingredients (sanitized onion or celery, or 20% vinegar-based product) were inoculated with 2 log CFU/g Pantoea/Erwinia, *Erwinina persicina*, *Erwinia* spp, or *Enterobacter pyrinus* isolated from celery (30°C, 3 d). Tuna salad preparations were also inoculated with a four-strain cocktail of *Morganella morganii*, previously isolated from raw scombroid fish (Mm; 18°C or 30°C, 3 d). Plate counts and MPN-PCR were performed on all inoculated samples; Hst was determined fluorometrically.

Results: HPB from celery were 0.7-4.3 log CFU/g higher in the presence of sanitized celery and onions vs plain tuna salad; only *E. pyrinus*produced significant Hst levels (513-2046 ppm; 30°C, 3 d). Mm increased by 2-3 log CFU/g in plain tuna salad and tuna salad with onion and by 4-5 log CFU/g in tuna salad with celery held at 18°C for 3 d. Mm produced 1315-3083 ppm Hst and increased by 2.5-4 and 5-6 log CFU/g in tuna samples after 1 and 3 days at 30°C, respectively. Vinegar-based product inhibited growth of all isolates by 1-5 log CFU/g.

Significance: Introduction of raw celery into commercially-prepared tuna salad can introduce HPB that may cause Hst poisoning if the product is temperature abused. We established that addition of a vinegar-based product to tuna salad can inhibit growth of HPB and/or production of Hst.

P1-75 Viability of *Salmonella* and *Listeria monocytogenes* in Delicatessen Salads and Hummus as Affected by Sodium Content

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Introduction: Health benefits that may result from reduction in sodium content of foods must be weighed against risks associated with promoting growth of foodborne pathogens. Of particular interest are high-a_w ready-to-eat foods. Delicatessen salads and hummus are examples within this microbiologically sensitive food category.

Purpose: The purpose of this study was to determine survival and growth characteristics of *Salmonella* and *Listeria monocytogenes*in mayonnaise-based deli salads and hummus as affected by sodium content.

Methods: Potato salad, macaroni salad, coleslaw and hummus (pH 5.07, 4.47, 3.94, and 4.59, respectively) were formulated to contain four concentrations of sodium: lowest (no sodium added), low (less than amount in control recipe), control (standard recipe) and highest (more than amount in control recipe). Test products were inoculated with *Salmonella* or *L. monocytogenes*. Populations of pathogens and total aerobic microorganisms were determined over a 27-day storage period at 4°C and 10°C.

Results: Populations of *Salmonella* (2.02 - 2.38 log CFU/g) decreased in coleslaw to undetectable levels (<1 CFU/25 g) within 13 days and in most formulations of macaroni salad within 20 - 27 days. Added sodium in macaroni salad stored at 4°C and hummus stored at 4 or 10°C appeared to protect *Salmonella* against inactivation. *L. monocytogenes*(1.86 - 2.23 log CFU/g) did not grow in test products but, with the exception of coleslaw containing sodium at a concentration used in the standard recipe, it was detected (> 1.0 log CFU/g) in all products stored at 4 or 10°C for 27 days. Sodium concentration did not significantly affect aerobic plate counts.

Significance: The acidic pH of deli salads and hummus is a major factor affecting viability of *Salmonella* and *L. monocytogenes*. In the absence of added sodium, death may be more rapid. Overall, however, the sodium content in selected deli salad and hummus recipes does not markedly affect the behavior of these pathogens.

P1-76 Occurrence of *Pseudomonas* spp. with Proteolytic Activity in Raw Goat Milk

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Introduction: *Pseudomonas* spp. are spoilage microorganisms present in milk and dairy products. Several strains are capable of multiplying under refrigeration temperatures, and to produce proteases and lipases. This behaviour indicates the necessity of proper control of this genus in refrigerated milk.

Purpose: The present study aimed to enumerate the total counts of *Pseudomonas* spp. in raw goat milk, and also their proteolytic colonies, in order to establish their percentage in this food product.

Methods: Sixty-one raw goat milk samples were collected from dairy goat farms located at Zona da Mata region, Minas Gerais State, Brazil, directly from milk cans and bulk tanks. All samples were ten-fold diluted in NaCl 0.85% (w/v) and plated on *Pseudomonas* agar plates added to sterile skim milk at 10% (v/v). All plates were incubated at 25°C for 3 days when all colonies were enumerated as *Pseudomonas* spp., and colonies with clear halos were enumerated as proteolytics. Selected proteolytic colonies from all samples were purified and subjected to biochemical tests to identify the genus *Pseudomonas*.

Results: Considering the obtained counts from *Pseudomonas* agar, it was observed a mean value of 3.9 log CFU/ml of supposed *Pseudomonas*, and 3.4 log CFU/ml of proteolytics. The percentages of proteolytic colonies, based on the total counts of *Pseudomonas* agar, varied from 0 to 100%: 11 samples (18.0%) presented proteolytics colonies at range of 90 to 100%, 7 (11.5%) between 80 to 90%, and 9 (14.8%) between 50 and 80%. A total of 496 proteolytic colonies were subjected to biochemical tests, and 106 were identified as *Pseudomonas* spp., representing the presence of this genus with proteolytic activity in 33 (54.1%) raw goat milk samples

Significance: The results obtained in the present study indicated the relevance of proteolytic *Pseudomonas* spp. as constituent of the raw goat milk microbiota, leading to further studies to characterize their proteolytic potential and activity.

P1-77 Optimization of Formula of NaCl, pH and a_w in Low-salt Soy Sauce against *Zygosaccharomyces rouxii* Using Experimental Mixture Design

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Introduction: Processed foods with low contents of salt have been highly required due to current trend of healthy diet. Non-acid sauces that contain reduced salt have difficulties to meet requirements not only for better qualities but also stable shelf life at room temperature at the same time. However, it could be possible to solve those defects effectively by optimization of microbial growth factor formula like pH, a_w, salt contents simultaneously with applying statistical experimental method.

Purpose: Purpose of this study was development of statistical regression model against *Zygosaccharomyces rouxii* in low-salt soy sauce by optimization of 3 kinds of key microbial growth factor such as NaCl, pH, a_w using experimental mixture design to achieve stable shelf life at room temperature.

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Methods: Target product was low-salt soy sauce distributed at room temperature and target strain was *Zygosaccharomyces rouxii* KCCM11300 which is the most resistant to NaCl, a_w and pH out of tested strains. Strain was cultured in broth of low-salt soy sauce adjusted with NaCl (for NaCl 3.2~5.2%), acetic acid (for pH 4.0~5.0) and glycerol (for a_w 0.88~0.93) for 4, 7, 10, 14 days and enumerated their loads in each day. The experimental design was applied using Design Expert® 8.0 software.

Results: Model equation was obtained to optimize inhibitory interaction against *Zygosaccharomyces rouxii* KCCM11300 by adjusting NaCl, pH and a_w. According to this model, *Zygosaccharomyces rouxii* KCCM11300 could be inhibited in the condition of NaCl 5.0 % with pH 4.27, a_w 0.900 and with pH 4.30 and a_w 0.891, respectively, that it had been recorded same inhibitory pattern only using 15% of NaCl. Under these conditions, experimental data was closely identified with predicted model. Applied model was cubic mixture model with 0.94 of R^2.

Significance: By using proposed equation, it could be highly expected that microbial spoilage in non-acid and low-salt sauces can be protected effectively to provide long shelf life at room temperature. This approach to reduce spoilage in low-salt sauces might be applied to develop other liquid types of foods as well as soy sauces.

P1-78 Good Manufacturing Practices: Knowledge of Food Handlers, Perception of Consumers and Its Adoption in Foodservice Restaurants

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Introduction: In Brazil, more than 40% of the > 4,500 foodborne disease outbreaks (FDO) notified among 1999-2008 occurred in restaurants/foodservices. The effective implementation of good hygienic practices (GHP) may ensure that consumer's expectations on food safety are satisfied and contribute for reduction of FDO associated with foodservices.

Purpose: The purpose of this study was to evaluate the awareness of food handlers regarding the implementation of GHP in foodservices and to compare it with the perception of consumers on hygienic conditions and with the GHP implementation.

Methods: The study was performed in two foodservices located in cities of the state of Sao Paulo, Brazil. The knowledge of food handlers (n=15) on GHP was the first step studied through the application of a check-list comprising 35 questions. Then, the perception of consumers (n=72) on hygienic practices of the foodservices was evaluated after the application of a check-list with 12 questions. The final step consisted of the assessment of GHP implementation through the use of a check-list developed by the Brazilian National Surveillance Sanitary Agency. The results were tabulated and percentages of conformity were calculated.

Results: Ninety seven percent of food handlers in both foodservices responded they were aware of the basic principles of GHP. At least > 40% and > 50% of consumers in foodservices A and B, respectively, considered these establishments as "high level" regarding each section of GHP questionnaire. Non-conformities in GHP implementation in foodservice A were observed in the sections of raw materials (16%) and exposure of foods for consumption (14%). On the other hand, foodservice B presented an average of 44%, 16% and 5% of non-conformities in equipment/facilities, raw materials and food preparation sections, respectively.

Significance: The study provides insights on how GHP is viewed by food handlers and consumers of two foodservices, followed by the validation of perceived view through assessment of GHP implementation.

P1-79 *Withdrawn*

P1-80 Vinegar as a Sanitizing Agent for Leafy Greens on Small Farms

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Developing Scientist Competitor

Introduction: Small farms (revenue < $50,000/month) are not subject to USDA regulations regarding post-harvest practices and therefore have no set standards on washing produce. Fresh produce has been implicated in multiple foodborne illness outbreaks over the past decade. The use of effective sanitizers for washing produce may help reduce microbial contamination and increase shelf life.

Purpose: The objective of this research was to examine the sanitizing effects of white distilled vinegar solutions to improve the quality and shelf-life of leafy greens by enumerating aerobic microorganisms, coliforms, *Escherichia coli*, yeast, and mold.

Methods: To determine the efficacy of vinegar as a sanitizing agent we obtained mixed greens and romaine lettuce samples from local farmers. Vinegar solution (1.25% and 2.5% acetic acid) was prepared in a salad spinning device for home use. The lettuce was submerged for 30 sec, spun for 45 sec and allowed to air dry for 1 min. Each sample was stomached with sterile 0.1% peptone, diluted and plated on appropriate media. Aerobic Plate Counts (APC), *E. coli*/coliforms, and yeast and mold were evaluated for the samples. The microbial counts on the lettuce were evaluated pre- and post-intervention.

Results: The vinegar solutions of 1.25% and 2.5% acetic acid reduced APCs on mixed greens by ~1 log CFU/g and on romaine by ~2 log CFU/g. The *E. coli* and coliforms counts were reduced on mixed greens and romaine by ~0.5 log CFU/g and ~1.75 log CFU/g, respectively. The intervention reduced yeast and mold counts by ~2 log CFU/g and ~1.5 log CFU/g on both mixed and romaine lettuce samples, respectively.

Significance: These results demonstrate that vinegar can reduce microbial contamination and possibly improve the shelf-life of leafy greens. Furthermore, this can help small farmers who may not have the means to use industrial washers to sanitize fresh produce.

P1-81 Spoilage Classification Models Using Metabolomics and Fingerprinting

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Introduction: Sensory and microbiological analyses are most often used to evaluate the freshness, spoilage or safety of meat and meat products. The disadvantages of sensory analysis, despite being the most acceptable and appropriate method, is its reliance on highly trained panelists, which makes it costly and unattractive for routine analysis.

Purpose: The aim of this work is to develop classification models for accessing freshness (e.g., microbiological and organoleptic parameters) in beef filet samples using metabolomics and fingerprinting with conventional (HPLC) and non-destructive (FTIR, e-nose) instrumentation using support vector machines (SVMs).

Methods: In the framework of SYMBIOSIS-EU project, the shelf life of beef filets stored aerobically at 0, 5, 10, 15 and 20°C was investigated. The microbial association of meat and the temporal biochemical changes were monitored. Microbiological analyses, including total viable counts, pseudomonads, *Brochothrix thermosphacta,* lactic acid bacteria, and *Enterobacteriaceae*, were undertaken, while in parallel sensory assessment, pH measurement, HPLC analysis of the organic acid profiles, FT-IR, and eNose measurements were recorded and the data were analyzed.

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Results: The data derived from HPLC, e-nose and the fingerprint from FTIR were used to develop two sets of SVM models: (i) based on individual and (ii) combined datasets using all possible pairwise combinations, in order to assess the effect of combined data in improving the prediction performance of the developed models. Model performance was assessed using independent subsets. The models were then optimized to achieve the best classification accuracy for specific data types or data type combinations.

Significance: The significance of these findings relies on the fact that such approaches can provide reliable indication of the quality status of meat in retail regardless of whose perspective you take, i.e., that of the consumer, the industry, the inspection authority, or the scientist.

P1-82 Persistence of Human Norovirus in Simulated Gastric Fluid

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Introduction: Human noroviruses (HuNoV) are responsible for a significant amount of foodborne disease. Contamination of food with fecal material is well recognized, but HuNoV are also shed in vomitus. The importance of vomitus in foodborne transmission of HuNoV is currently unknown.

Purpose: The purpose of this project was to compare the persistence of HuNoV suspended in vomitus [using simulated gastric fluid (SGF), pH 2.5] to that suspended in phosphate buffered saline (PBS, pH 7.4).

Methods: A representative outbreak HuNoV strain (GII.4) was suspended in SFG or PBS, and the same solution inoculated on stainless steel coupons. The suspensions and coupons were held at room temperature for up to 42 days, with periodic recovery of virus inoculum. Viral RNA was extracted and quantified by RT-qPCR targeting the *orf* 1-*orf* 2 junction, with and without a prior RNase pre-treatment.

Results: There were no statistically significant differences ($P > 0.05$) between virus persistence over time when comparing RT-qPCR assays with and without prior RNase treatment except for when HuNoV was diluted in PBS and used in surface studies. In suspension assays, the genome copy number of HuNoV GII.4 suspended in SGF decreased by 1.0 log over 42 days, while that for the virus suspended in PBS dropped by 0.1 log. When inoculated on stainless steel coupons, the HuNoV GII.4 genome copy number decreased by 0.03 log in 42 days for inoculum diluted in SGF, while a decrease of 2.2 log was observed for the PBS inoculum. In both cases, there were statistically significant ($P < 0.05$) differences between virus persistence in SGF when compared to PBS.

Significance: This study demonstrates that HuNoV suspended in vomitus-like material can persist for long periods. Such environmental persistence likely contributes to foodborne transmission, as contaminated surfaces serve as a source of contamination to foods, especially during storage or preparation.

P1-83 Genomic Attributes Associated with Host Adaptation in *Campylobacter jejuni* and *Campylobacter coli* from Poultry and Swine

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Introduction: *Campylobacters* are zoonotic bacteria frequently colonizing meat animals and are associated with significant foodborne disease burden. Population analysis data suggest pronounced genetic diversity among *Campylobacter jejuni*and *Campylobacter coli*, the two species predominantly contributing to human diarrheal disease. Current evidence suggests potential for host-association attributes, especially in *C. coli*.

Purpose: The objective of our study was to employ genome sequence information and functional genetic analyses in order to identify genomic attributes associated with animal host preference in *C. jejuni* and *C. coli*.

Methods: Total genomic DNA of one turkey-derived *C. jejuni*, two turkey-derived *C. coli* and one swine-derived *C. coli* was sequenced. Coding sequences were analyzed in terms of their presence in the different sequenced genomes using a novel functional genome distribution algorithm followed by a custom cluster comparison. Presence and comparative genomic location of homologous sequences in the different genomes was determined. A membrane macroarray-based hybridization format was used to assess host specificity of putative host-associated ORFs. Primers derived from these ORFs were used as probes to hybridize a panel of 20 strains of *C. coli*from different animal hosts.

Results: Comparison of the two turkey-derived genomes revealed 387 and 83 ORFs unique to *C. coli* 11601 and 6067, respectively. Comparative analysis of ORFs conserved in the turkey-derived *C. coli* strains but absent from *C. coli* 6461 (swine) identified 17 putative turkey-associated candidates. Similarly, we identified 82 putative host-associated ORFs harbored by *C. coli* 6461 (swine) but absent from both of the turkey-derived genomes, including a putative DNA methyltransferase. Other sequences with potential for host association seem to be due to insertion events in the genome.

Significance: The genome sequence data suggest that the majority of strain-specific sequences in the two sequenced turkey-derived strains have a highly conserved homolog among other campylobacters, suggesting horizontal gene transfer among members of the *Campylobacter* pangenome.

P1-84 The Addition of Putative Virulence Markers to an Established P-BIT Typing Scheme Enables *Campylobacter coli*, but not *Campylobacter jejuni*, to be Separated into Country of Origin

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Introduction: *Campylobacter* is the leading cause of foodborne bacterial gastroenteritis in many industrialised countries. Standardized protocols for Multi Locus Sequence Typing exist to facilitate the epidemiological study of these organisms. A PCR binary typing system (P-BIT) recently developed in New Zealand provides a less expensive, rapid and highly portable typing system.

Purpose: The purpose of this study was to determine if the P-BIT system with or without additional data can differentiate *Campylobacter*from three countries.

Methods: A total of 58 *Campylobacter* isolates from Australia (15 *C. jejuni*, 5 *C. coli*), Poland (16 *C. jejuni*, 6 *C. coli*), and Malaysia (9 *C. jejuni*, 7 *C. coli*), isolated from chicken carcasses at the end of processing or at retail, were examined for the presence of the 18 P-BIT genes and eight putative virulence markers. P-BIT and gene presence were analysed using Bionumerics with simple matching and UPGMA. Clusters were separated at the 75% level.

Results: Across countries only a single pair of isolates from Australia and Poland had the same P-BIT code. Within countries four pairs of Australian and a single pair of Malaysian isolates had the same P-BIT code. Including a further eight genes involved in various aspects of

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pathogenicity in analysis of P-BIT data allowed separation of *C. coli* from *C. jejuni* and allowed further separation by country. Australian isolates had a significantly higher prevalence of *ciaB* while Polish isolates had a significantly higher presence of the *cdt*gene cluster.

Significance: The P-BIT method in conjunction with other putative virulence genes may be able to differentiate *C. coli* but not *C. jejuni* based on geographic origin.

P1-85 Survival of *Staphylococcus aureus* on Dried Fishery Products as a Function of Temperature

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Developing Scientist Competitor

Introduction: Among the imported and domestic dried fishery products at retail market in Korea, 33.8% was reported to be contaminated with *S. aureus*. Dried fishery products were recommended to be kept at 10°C for quality maintenance of the products at retail markets, but were very often sold at ambient temperature in convenience stores and traditional Korean markets.

Purpose: The object of this study was to evaluate survival of *S. aureus* and production of Staphylococcal enterotoxin A on dried filefishes and julienned squid at 10, 24, and 35°C.

Methods: Dried filefishes and julienned squid were inoculated with enterotoxin A producing *S. aureus* at a concentration of 10^6CFU/g, respectively, and packed into polyethylene bags for storage at 10, 24 and 35°C. At selected times after inoculation, the diluted samples were plated onto Baird-Parker agar in duplicate, and incubated at 35°C for 48 h. Production of Staphylococcal enterotoxin A in dried fishery products was analyzed using a TECRA SE visual immunoassay kit and ELISA reader.

Results: *S. aureus* on both dried filefishes and julienned squid survived longer at 10 °C than 24 or 35°C. After 5 months storage, populations of *S. aureus* resulted in reduction of 1~1.7 at 10 °C and 5~5.5 log CFU/g at 24°C. At 35°C, *S. aureus* was undetectable on dried filefishes and julienned squid after 14 and 16 days, respectively. Toxin production was not increased during 5 months, regardless of storage temperature. Survival kinetic parameters of the observed data were also well fit to the Weibull equation (GIna FiT v1.5, R^2>0.95). Shape(P) and scale(delta) values of the Weibull equations were further described in a secondary model as a function of temperature using second-order polynomial model.

Significance: The quality of dried fishery products was better maintained at 10°C. However, the low storage temperature has more potential for *S. aureus* survival on dried fishery products than ambient temperatures and thus causes a potential safety risk of the dried fishery products.

P1-86 Distribution of Pathogenicity Islands in Shiga Toxin-producing *Escherichia coli*

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Developing Scientist Competitor

Introduction: Shiga toxin–producing *Escherichia coli* (STEC) can cause severe foodborne diseases. The genetic basis of STEC virulence is not fully elucidated. It is speculated that horizontal gene transfer and acquisition of pathogenicity islands (PAIs) play a major role in the evolution of STEC pathogenicity.

Purpose: To determine the distribution of PAIs OI-122, OI-43/48, OI-57 and high pathogenicity island (HPI) in STEC.

Methods: STEC were classified into seropathotypes A to E based on reported occurrence in outbreaks, diarrhea and severe diseases. Specific PCR assays were used to identify *eae*, *stx* and additional virulence genes located on PAIs (OI-122: *pagC*, *sen*, *efa-1*, *efa-II, nleB* ; OI-43/48: *treC*, *ureC*, *iha. aidA-1*, OI-57: *nleG2-3*, *nleG5-2, nleG6-2*; HPI: *fyuA, irp2*).

Results: The prevalence of OI-122 and OI-57 were significantly higher in seropathotypes associated with server diseases and outbreaks than other seropathotypes ($P < 0.0001$). HPI was missing in seropathype A and distributed evenly in seropathotypes B, C, D and E. Most virulence genes located on OI-122, OI-43/48 and OI-57 were significantly more prevalent in seropathotypes linked to severe disease and outbreak than other seropathotypes ($P < 0.0001$). OI-122, OI-57 and OI-43/48 and their associated virulence genes, except *pagC* and *iha,* were found highly associated with *eae*-positive STEC strains, while HPI mostly occurred independently of *eae* presence. The β-glucuronidase-negative *E. coli* O157:H7 strains carried complete OI-122 and OI43/48, whereas β-glucuronidase-positive *E. coli* O157:H7 strains only contain parts of these PAIs.

Significance: These findings suggest that OI-122, OI-57 and OI-43/48 but not HPI are highly associated with *eae*-positive STEC strains and could contribute to STEC virulence. Virulence genes in PAIs that are associated with severe diseases can be used as molecular markers to identify highly virulent STEC in the food industry and public health labs.

P1-87 Occurrence of *Campylobacter* spp. in Dairy Cattle Farms of Quebec, Canada

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Introduction: Although consumption of poultry meat is recognized as a major source for human campylobacteriosis, the origin of infection remains unknown in many cases. New and emerging reservoirs of *Campylobacter*, like cattle, are now being considered in the epidemiology of this disease. Currently, very little data on the prevalence of *Campylobacter*in dairy herds are available in the province of Quebec.

Purpose: The objective of this study was to evaluate the presence of *Campylobacter*in Quebec dairy herds.

Methods: Forty farms were visited once during the summer of 2011. A total of 800 fecal samples were collected from all farms by sampling twenty lactating cows per herd (one sample per cow). Water provided to animals, milk from bulk tank and fecal matters from replacement animals' pens were also analyzed for the presence of *Campylobacter*. The bacterial identification was performed by PCR, targeting the 16S rRNA gene, from isolated colonies. Information regarding farm management was collected using a short survey.

Results: In total, 29 farms were found positive for *Campylobacter* spp. with *C. hyointestinalis* being the most prevalent species (68%). *C. jejuni* was found in 20 farms and accounted for 24% of the total *Campylobacter* species isolated from lactating cows. Among herds found *Campylobacter* negative in all lactating cows, feces from replacement animals were also found to be *Campylobacter* negative 85% of the time. No *Campylobacter* were recovered from water or milk samples. Among the farm management practices evaluated, hand-feeding of the animals was associated with the presence of *C. jejuni* on farms ($P = 0.00812$).

Significance: This study is the first one to report the presence of *Campylobacter* in dairy cattle herds of Quebec, a province where dairy production is very important. Identification of other reservoirs of *Campylobacter* may be useful for understanding the ecology of *Campylobacter*.

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P1-88 CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) as a Potential Molecular Subtyping Marker for High-risk Shiga Toxin-producing *Escherichia coli* (STEC) Isolates

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a subgroup of *E. coli*strains that causes serious public health concerns due to their ability to cause disease of varying severity from mild diarrhea to bloody diarrhea to hemolytic uremic syndrome (HUS). For prevention and control purpose, it is important to be able to rapidly detect and track high-risk STEC strains. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an immune system which confers to bacteria resistance to phages and plasmids by the acquisition of short DNA sequences (spacers) that target the invading DNA.

Purpose: Previously, we have shown that CRISPR sequences are effective subtyping markers for *Salmonella enterica* subsp. *enterica*. Here, we hypothesize that the CRISPR loci of STECs can be potential markers for high-risk STEC isolate detection.

Methods: The CRISPR1 locus (*cas* to *iap*) was amplified and sequenced from a collection of 53 STEC isolates including 6 high-risk O serogroups (O26, O103, O111, O121, O145 and O157) from both human and non-human sources. Sequences were analyzed using a self-developed R script and each unique spacer was assigned a number. The spacer array of each isolate was represented by the spacer numbers.

Results: There were 5, 9 and 5 unique spacer arrays observed in O26, O103 and O111. Despite overlap in spacer content, only 3 complete spacer arrays were shared between O26 and O103 and 2 were shared between O103 and O111. No spacer array was common to all three serogroups. For the O serogroups that are less frequently observed clinically, the spacer found were distinct from those seen in O26, O103, and O111 isolates.

Significance: Therefore, CRISPR may be an effective molecular subtyping marker for certain STEC serogroups.

P1-89 Comparative Genomics of *Salmonella* Phage Diversity on Dairy Farms: Distinguishing the "Good" from the "Bad"

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Developing Scientist Competitor

Introduction: *Salmonella* is a foodborne pathogen that is widely distributed among livestock. *Salmonella* on dairy farms is a concern because it can be found at high prevalence and in the absence of clinical signs, underscoring the difficulty in recognizing infected herds. *Salmonella* phages can act as predators of *Salmonella* (killing *Salmonella*, the "good"); however they can also act as a vector for horizontal transmission of genes (the "bad").

Purpose: The purpose of this study was to assess the genomic diversity and putative roles of lytic and lysogenic phages on dairy farms.

Methods: The genomes of 22 *Salmonella* phages from nine dairy farms with a history of *Salmonella* isolation were fully sequenced. Comparative analyses of phage genomes isolated from the farms and previously sequenced phage genomes were conducted. In addition, phylogenetic analysis was performed to classify the phages.

Results: The *Salmonella* phages isolated from farms are highly diverse and can be classified into nine different groups, representing 13 lysogenic and nine lytic phages. We found that 9/22 of the phages represent types of *Salmonella* phages that have not previously been reported. In addition, we identified phage-borne virulence (adhesins, *msgA*) and antimicrobial resistance (tellurite and sulfonamide resistance) genes. Phages from the same group were found on farms hundreds of miles apart. Finally, lytic phages isolated from farms with a high prevalence of *Salmonella* (9-50%) were more likely to infect the most prevalent *Salmonella*serovar present on that specific farm.

Significance: This represents the first comprehensive study on the genomic diversity of *Salmonella* phage isolated from dairy farms. The high diversity of lytic and lysogenic phages on farms and the presence of phage-borne resistance genes in some of the sequenced phages suggest that *Salmonella* phages play an important role as both predators and drivers of *Salmonella* evolution.

P1-90 The Spread of *Cronobacter sakazakii* in the Domestic Kitchen

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Introduction: *Cronobacter sakazakii*is an emerging, opportunistic pathogen implicated in severe meningitis, sepsis, and necrotizing in premature and full-term infants. Being widely distributed, this pathogen has also been detected in processing environment, and domestic environments; thereby posing some level of safety risk to infants and individuals with low immunity.

Purpose: The purpose of this study was to assess the occurrence of *C. sakazakii*in selected domestic kitchens. In addition, the antimicrobial resistance patterns of the pathogen were also examined for points of public health significance.

Methods: A total of 234 contact sites in seventy-eighty domestic kitchens were analyzed and tested for *C. sakazakii.* Consumers used dish clothes and cleaning sponges were also tested for the pathogen. Chromogenic Cronobacter Druggan-Forsythe-Iversen (DFI) agar, API 20E test system, and PCR were used for isolation and identification. *C. sakazakii* was identified by PCR targeting the 16S-23S ribosomal DNA and the *OmpA* genes. Antimicrobial susceptibility was determined using the disk diffusion method.

Results: *C. sakazakii* was recovered from 26.9% of domestic kitchens visited. *C. sakazakii* isolates demonstrated multidrug resistance where the highest resistance was observed in penicillin (76.1%), followed by tetracycline, (66.6%), ciprofloxacin (57.1%), and nalidixic acid (47.6%). None of the tested *C. sakazakii*was resistant to gentamycin.

Significance: This study suggests that antibiotic-resistant *C. sakazakii* could be present in various sites of domestic kitchen.

P1-91 Survival of *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes* during Storage of Fermented Black and Green Table Olives

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Introduction: Table olives can be considered as ready-to-eat (RTE) products for which there is an increasing rate of production and consumption worldwide, while foodborne incidence regarding RTE products is more often reported. Although these RTE products are fermented and as such can be considered safe, available information regarding the pathogenic behavior due to cross contamination in these foods is limited.

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Purpose: To monitor the survival of three pathogenic bacteria during storage of fermented table olives, approaching the cross-contamination scenario during the packaging process of olives.

Methods: The survival of *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes*was studied during the storage of fermented green table olives in brine at 20°C, and fermented black table olives in air at 4 and 20°C. Each pathogen was tested as a mixed culture (3 or 5 strains of each pathogen were used for black or green olives, respectively).

Results: In green olives packed in brine, the population of *E. coli* O157:H7 and *S.* Enteritidis was reduced gradually and detected until the 19th and 27th day of storage, respectively, while *L. monocytogenes* could be detected until the end of storage period (48 days). The population of all pathogens in aerobically packed black olives showed a remarkable decrease below the detection limit after the first day of storage, at both storage temperatures. *E. coli* O157:H7 and *S.* Enteritidis could not be detected after 5 and 6 days of storage, respectively, at both temperatures. The population of *L. monocytogenes*could not be detected after the 6th day of storage at 20°C, but almost all samples at 4°C were found enrichment positive at the end of storage.

Significance: The results showed that successfully fermented table olives generally do not support the growth of pathogens. However, the adaptation and survival of pathogens is possible and for this reason risk-assessment studies and application of strictly good manufacturing practices are needed to reduce the possibility of cross contamination.

P1-92 Spray Intervention Treatments and Cooking to reduce *Escherichia coli* O157:H7 and Non-O157 STECs on the Surface of Beef Subprimals, and within Needle-Tenderized Steaks

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Developing Scientist Competitor

Introduction: In recent years, the reported cases of illness caused by non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) have been rising, becoming a threat to public health, and leading to six non-O157 STECs to be declared as adulterants in ground beef by the USDA-FSIS.

Purpose: The objectives were to (i) compare the difference of antimicrobial spray treatments on the reduction of *E. coli* O157:H7 and non-O157 STECs on beef subprimals after 14 days during vacuumed, refrigerated storage, and to (ii) evaluate the effect of cooking to reduce *E. coli* O157:H7 and non-O157 STECs (Serotypes O26, O103, O111 and O145) in needle tenderized meat.

Methods: USDA Select beef strip loins were inoculated with 10^6 or 10^2 cfu/cm^2 cocktails of either *E. coli* O157:H7 (study 1) or non-O157 STECs (study 2) before spray treatments [water, 5% lactic acid (LA), 0.02% hypobromous acid (HB2), or 0.02% peroxyacetic acid] and vacuumed storage. On day 14, each subprimal was needle tenderized, cut into three 2.54 cm-thick steaks, and assigned to uncooked, 50°C, and 70°C internal temperatures. Swab samples (50 cm^2) were collected from the subprimal surface after inoculation, five minutes after spray treatment, and before tenderization. An additional swab was obtained from the surface of one raw steak per treatment. Internal meat samples (10 grams) were also taken from all uncooked and cooked steaks.

Results: After 14 days, *E. coli* O157:H7 was reduced by LA with 2.3 $\log_{10}$ cfu/50 cm^2, while non-O157 STECs was reduced by HB2 with 1.0 $\log_{10}$ cfu/50 cm^2. Regardless of spray treatment, lower cooking temperature did not significantly reduce the non-O157 STECs, with serotype O103 being the most prevalent strain found in cooked samples. Cooking to 70°C was effective in controlling O157:H7 to non-detectable numbers.

Significance: The results of this study provide the information about non-O157 STEC that can be used to develop more efficient and effective subprimal intervention strategies aimed to prevent STEC in non-intact meat products.

P1-93 Comparative Genomics Analysis of *Salmonella* Pathogenicity Islands (SPI-5 and SPI-6) of *Salmonella* Newport

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Developing Scientist Competitor

Introduction: *Salmonella* Newport is the third most common *Salmonella* serotype associated with foodborne outbreaks in the US. Whole genome sequencing and comparative genomics analysis are useful tools to provide a better understanding of microbial pathogenicity and evolution. *Salmonella* pathogenicity islands 5 and 6 (SPI-5 and SPI-6) are important for *Salmonella* enteropathogenicity.

Purpose: Comparative genomics analysis of SPI-5 and SPI-6 were performed to study *S.*Newport genomic variation and evolutionary history.

Methods: Shotgun 454 pyrosequencing was employed to 24 *S.* Newport strains from various sources and geographic locations. Other five serotypes (*S.* Hadar, *S.* Saintpaul, *S.* Paratyphi C, *S.* Choleraesuis, *S.* Virchow) were selected to serve as outgroup genomes for comparison. GARLI 2.0 was selected to perform phylogenetic analysis and MEGA5. Mauve was used for comparative genomics analysis.

Results: The 24 *S.* Newport strains displayed clear geographic structure, splitting into two major groups, *S.* Newport Lineages II and III. SPI-5 was present in all strains. In several strains, SPI-5 contained 40 kp insertions associated with bacteriophages that were acquired through horizontal gene transfer. For example, Insertion-1 was located between tRNA-ser and pipA loci within SPI-5 among seven strains sharing a common ancestor. Independently, Insertion-2 was found in strain 198_shrimp_India at the same loci. SPI-6 was also present in all strains except 198_shrimp_India, 201_squid_Vietnam, 202_pepper_Vietnam and *S.* Virchow SL491. The four genomes shared one common sequence consisting of 25 genes between tRNA-asp and sinR loci without SPI-6, namely, Insertion-3. These three *S.* Newport strains isolated from Asia (198_shrimp_India, 201_squid_Vietnam and 202_pepper_Vietnam) were grouped into a subgroup with strain 203_pig_ear_CA. All strains (n=18) from North America contained SPI-6 at the same loci. In addition, *tcf* fimbrial operon was identified downstream of *sinR* in 201_squid_Vietnam, 202_pepper_Vietnam, *S.* Virchow SL491 and *S.* Choleraesuis SC-B67.

Significance: Whole genome sequencing enables us to perform comprehensive genomics analysis of foodborne pathogens. Our data suggested that *S.* Newport strains have extensive variation within SPIs, and that horizontal gene transfer appears to play a critical role in the evolution of these significant pathogenicity determinants.

P1-94 Evaluations of Three Multiplex Real-Time PCR Assays for the Detection of Six Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups and the *eae*, *stx1*, and *stx2* Genes in Ground Beef and Trim Enrichments

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Introduction: Regulations in the United States starting in June 2012 will require industry to begin monitoring for six Shiga toxin-producing *E. coli* (STEC) serogroups. The regulations will specifically require detection of the so called "big-six" STEC (O26, O45, O103, O111, O121, and O145) as these have been most frequently associated with outbreaks of foodborne illnesses in the United States. There is an additional requirement related to the detection of the virulence genes *eae,* $stx_{1/2}$.

Purpose: The purpose of this study was to determine the feasibility and conduct initial evaluations of three separate, previously developed, multiplex PCR assays for the detection of the big-six serotypes of STEC and the *eae,* $stx_{1/2}$ genes in spiked food enrichments (ground beef and trim).

Methods: The three assay configurations were: Assay 1 – O26, O111, O121, and internal positive control (IPC); Assay 2 – O45, O103, O145, IPC; Assay 3 – *eae,* $stx_{1/2}$, IPC. Both 65 g and 375 g ground beef and 375 g trim enrichments were diluted (1:10 or 1:5) in pre-warmed (44°C or 46°C) sterile tryptic soy broth (TSB) and/or TSB + 2 mg/l novobiocinand incubated for 9-24 h at 41 °C. Twenty µl was removed for processing and testing by the BAX® System methods.

Results: For 65 g ground beef samples all spiked targets (*n* = 18) were detected appropriately in as little as 9 h of enrichment. For 375 g ground beef samples all spiked targets were detected (*n* = 30) appropriately within 12 h of enrichment. For 375 g trim samples all spiked targets (*n*= 18) were detected appropriately within 10 h of enrichment.

Significance: These results demonstrate the feasibility of deploying a panel of three novel real-time PCR assay configurations for the detection and monitoring of STEC O groups as well as the virulence genes, *eae,* $stx_{1/2}$ in actual food enrichments. Studies with other food types including produce are ongoing.

P1-95 Optimization and Characterization of an Integrated Cell Culture-PCR Assay for the Detection of *Coxiella burnetii* Nine Mile in Whole Milk

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Developing Scientist Competitor

Introduction: *Coxiella burnetii* (*Cb*), a heat-resistant, obligate intracellular bacterium, was used historically as the reference organism to determine proper milk pasteurization conditions. An *in vitro* Integrated Cell Culture-PCR (ICC-PCR) assay may be useful as a substitute for animal bioassays for evaluating *Cb*inactivation in novel dairy products and processes.

Purpose: To optimize and characterize an ICC-PCR assay for detection of viable *Cb*in whole milk.

Methods: In order to optimize *Cb* infection, confluent Vero cell monolayers were infected for 2, 24, and 48 h with *Cb* resuspended in whole milk. Infected cells were then supplied with fresh RPMI media and further incubated for 9 days to encourage *Cb* propagation. The ability of *Cb* in whole milk to propagate in Vero cells was evaluated by diluting *Cb* in either whole milk or tissue culture media followed by 48 h infection and 9-day propagation. The level of *Cb* propagation was determined by subtracting the Day 9 post-infection *Cb* ge/ml from the Day 0 post-infection level. Replicate samples were performed for each inoculation level. In all cases, infected cells were subjected to freeze-thaw followed by spin column DNA extraction and amplification using a TaqMan-MGB qPCR assay based on published primers for the *Cb*IS1111a transposase gene.

Results: From three independent trials, the optimal infection period was determined to be 48 h with an approximate 0.5 log increase in propagated levels over cells infected for only 2 h. In addition, even with extended infection time of 48 h, no visible monolayer damage was noted. The detection limit was 13 cells/ml in whole milk, regardless of diluent. The maximum level of propagation in whole milk as determined by qPCR was 2 log ge/ml. When resuspended in RPMI + 1% FBS, *Cb* levels increased 2.5 – 3 log ge/ml after 9 days propagation. Background signals were negligible.

Significance: This optimized ICC-PCR assay allows detection of infectious *Cb* in whole milk, and may be used to study factors affecting *Cb* inactivation during processing.

P1-96 Motility of Filamentous Cells of *Salmonella enterica* Serovar Enteritidis E40

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Introduction: Previous work from our laboratories has shown that exposure to increased salt concentration (7% w/v) results in substantial filament formation by *Salmonella* Enteritidis E40. These filaments have the ability to invade Caco-2 intestinal epithelial cells *in vitro*and are virulent upon intragastric inoculation into mice.

Purpose: During the course of our experiments, we noted that filamentous cells of *Salmonella* Enteritidis E40 were motile. The purpose of this study was to compare the motility of filamentous and non-filamentous forms of*Salmonella*Enteritidis E40, and to assess the effects of medium and time of incubation on motility.

Methods: *Salmonella* Enteritidis E40 was grown on trypticase soy agar (TSA) (control, non-filamentous) and TSA + 7% NaCl (filamentous) at 30°C for 4 days. The bacterial cells were harvested and resuspended in Caco-2 tissue culture medium (DMEM with supplements) with 10 % fetal bovine serum. The cells were then incubated in the same medium for up to 6 hr at 30°C in a CO_2incubator. Every 30 min, a sample was removed and plated on swimming agar (0.25% final concentration). The plates were then incubated at 37°C for 7 hr. At that time the diameter of the bacterial lawn (i.e., motility) was measured and expressed in cm. Samples removed from the Caco-2 medium at the same time points were also used to estimate colony forming units, by dilution in saline and plating on sheep blood agar.

Results: Filamentous cells of *Salmonella* Enteritidis E40 exhibited substantially greater motility than non-filamentous cells. For example, filamentous cells incubated in DMEM with 10% FBS for 150 min migrated approximately 8 cm on swimming agar, whereas non-filamentous cells migrated approximately 4.4 cm. During a 4-hour incubation in Caco-2 medium, an initial inoculum of 40 µg/ ml wet weight non-filamentous cells increased a little more than one log (1.67×10^7 to 2.7×10^8 CFU) whereas in the same time frame the CFU of filamentous cells increased from 2.7×10^5 to 1.4×10^8CFU. Microscopic examination revealed that the greater increase in CFU for the filamentous cells reflects, in part, fragmentation of the filaments into smaller cells. Whether or not the medium contained fetal bovine serum seemed to have little effect on filamentous cell motility. However, there was greater motility of non-filamentous cells as the concentration of FBS increased. By 4 hours, most filamentous cells were broken up into smaller rods, resembling control cells, and swam faster than the control cells as evident by their large initial diameter in the swimming agar.

Significance: Filamentous cells of *Salmonella* Enteritidis E40 are highly motile, and can break up into many individual cells. This could facilitate spread of filamentous cells of *Salmonella* Enteritidis E40 in a contaminated environment.

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P1-97 Pentaplex Taqman Assay for the Detection of Pathogenic and Multidrug Resistant Strains of *Salmonella*

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Developing Scientist Competitor

Introduction: Overuse of antibiotics in the medical and animal industries is one of the causes for development of multidrug-resistant (MDR) food pathogens which are often difficult to treat. In the past few years, an increase in incidence of outbreaks caused by MDR *Salmonella* was documented. The ability to accurately and sensitively detect such pathogens in food is highly important.

Purpose: The objective of this study was to develop a rapid multiplex real-time assay for detection of pathogenic and antibiotic resistant *Salmonella*.

Methods: A pentaplex real-time PCR was designed targeting the virulence gene, invasin (*inv*), and four commonly found antibiotic resistance genes, *viz*. ampicillin, chloramphenicol, streptomycin and tetracycline. The sensitivity of the assay was determined via a standard curve with ten-fold serially diluted, 10 ng/µl to 1 fg/µl, genomic DNA. To avoid false negative results and to increase the reliability of the assay, an internal amplification control was added which was detected by a locked nucleic acid (LNA)-based probe.

Results: The assay was able to detect 200 genomic equivalents (1 genomic equivalents = 5 fg) of *Salmonella* DNA when single targets were tested, while in a multiplex format, the sensitivity of the assay decreased to 2000 genomic equivalents. The assay performed equally well on artificially contaminated samples of tomato, spinach, egg, ground turkey, ground chicken, chicken rinse, and ground beef of different fat contents (73:27, 80:20, 85:15 and 93:7). These food samples contaminated with 10 CFU/g could be detected after 12 h of enrichment with 100% reproducibility. The detection limit for unenriched samples was 10^4CFU/g.

Significance: The pentaplex real-time assay developed in this study can be applied as a sensitive and selective tool to detect antibiotic-resistant *Salmonella*, hence enhancing the safety of food.

P1-98 Lactic Acid Resistance of Non-O157 Shiga Toxin-producing *Escherichia coli* and Multidrug Resistant and Susceptible *Salmonella* as Compared to *Escherichia coli* O157:H7

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Introduction: Of recent interest to the meat industry is whether non-O157 Shiga toxin-producing *Escherichia coli* (nSTEC) and *Salmonella* are as sensitive to beef decontamination treatments as is *E. coli* O157:H7.

Purpose: This study compared the lactic acid resistance of individual strains of six nSTEC serotypes (O26, O45, O103, O111, O121 and O145; wild-type and spontaneous rifampicin-resistant variants), and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S*. Newport and *S*. Typhimurium, to that of a 5-strain mixture of *E. coli* O157:H7.

Methods: After inoculation (6 log CFU/ml) of a sterile 10% (w/w) beef homogenate, lactic acid was added to a target concentration of 5%. At 0, 2, 4, 6 and 8 min, aliquots were analyzed (two repetitions, three acid challenges per strain/mixture each) for survivors. Data were analyzed as a randomized complete block design.

Results: Results showed that in most cases, individual strains of wild-type and rifampicin-resistant variants of nSTEC, and antibiotic susceptible and resistant *S*. Newport and *S*. Typhimurium were less ($P < 0.05$) acid tolerant than the *E. coli*O157:H7 mixture of strains (wild-type and rifampicin-resistant). Inoculated wild-type and rifampicin-resistant *E. coli* O157:H7 strain mixtures (6.1 and 5.9 log CFU/ml, respectively) were reduced ($P < 0.05$) to 1.1 log CFU/ml and below the detection limit (<1.0 log CFU/ml) within 8 and 6 min, respectively. Out of the 35 nSTEC strains tested, 85.7% of the wild-type and 82.9% of the rifampicin-resistant variants reached the detection limit immediately after addition of lactic acid (0 min) or within 6 min of exposure. Similarly, 87.9% of the 33 *S*.Newport/Typhimurium strains reached the detection limit within 0 to 4 min, irrespective of serovar or antibiotic resistance phenotype.

Significance: The majority of the tested individual strains of nSTEC and the antibiotic susceptible and resistant *S*. Newport/Typhimurium showed lower acid resistance compared to *E. coli* O157:H7 indicating that these pathogens can be eliminated and/or reduced by lactic acid interventions at least as effectively as *E. coli* O157:H7 during beef slaughter operations.

P1-99 Inactivation of *Salmonella*, *Escherichia coli* O157:H7 and Non-O157 STEC by Hypochlorite Solutions with High Organic Loads

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Introduction: *Salmonella*, *Escherichia coli* O157:H7 and Non-O157 STEC have been recognized as foodborne pathogen concerns for fresh produce. Although chlorinated water (CW) is widely used in fresh produce processing to reduce pathogens and prevent cross-contamination, limited information is available on efficacy of pathogen reduction in chlorinated process water in the presence of high organic loads.

Purpose: In this study, inactivation of *Salmonella*, *E. coli* O157:H7 and Non-O157 STEC by CW (hypochlorite solutions) were evaluated for a range of free chlorine (FC) concentrations, contact times, and organic loads.

Methods: Two experiments were conducted. First, four strains of *Salmonella*, *E. coli* O157:H7 or Non-O157 STEC cells were separately inoculated into 12-well microplate containing FC (0-2 mg/l). Second, a multi-strain cocktail of pathogens was inoculated into 12-well microplates with fresh tomato extract (0-2.5%) or lettuce extract (0-2%), followed by CW (initial: 7-8 mg/l). In both experiments, after exposure times of 5, 10, 15, 30, 45, 60, 90, or 120 sec, 1-ml aliquots/per well were transferred into 96-well microplates with 2X tryptic soy broth plus 0.1% sodium pyruvate and dechlorination reagent. Pathogen survival was enumerated by spread plating onto XLT-4 or sorbitol MacConkey agar. Water quality, including pH, turbidity, chemical oxygen demand (COD) and free chlorine concentration, was tested. Statistical analysis of data was performed using PROC Mixed procedure of SAS.

Results: Water quality is highly impacted by the addition of tomato or lettuce juice extract as indicated by the rapid increase in water turbidity, COD, and decrease in residual free chlorine with the increase in extract concentration. This, in turn, significantly affects the survival of pathogens. In general, no survival of pathogens was detected in the solutions when the residual FC >0.5 mg/l for >30-sec contact time, or residual FC >1.0 mg/l and exposure time >5 sec. When the FC concentration and contact time were less than the above conditions, the survival of pathogens was strain dependant with the survival ranked as: *Salmonella* > *E. coli* O157:H7 > Non-O157 STEC.

Significance: These results show high organic loads severely and rapidly limit CW inactivation of foodborne pathogens.

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P1-100 Biofilms Improve Survival of *Salmonella enterica* vs. Tennessee in Dry Milk Powder and within a Simulated Gastrointestinal Environment

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Introduction: *Salmonella enterica* serovars have been linked to outbreaks associated with low a_w foods. The ability of biofilm forming pathogens, such as *Salmonella,* to survive thermal and chemical processes is improved; it is unclear if biofilms will also improve survival to desiccation and gastric stresses.

Purpose: To quantify the effect of physiological state (planktonic versus biofilm) and prior exposure to desiccation on *Salmonella* survival after passage through an *in-vitro*d igestion model.

Methods: *Salmonella enterica* Tennessee was grown as planktonic cells or as biofilms on glass beads and subsequently dried at room temperature and stored in dried milk powder (a_w = 0.3) for up to 30 days. *Salmonella* survival was quantified by serial dilution onto brilliant green agar before desiccation, after desiccation, after 1-day storage and after 30-day storage. At each sampling both lifestyles were tested for survival through a simulated gastrointestinal system.

Results: The physiological state and length of storage both affected the survival of *Salmonella* within the desiccated milk powder environment and after passage through an *in-vitro* digestion system (P < 0.01). Cells in a biofilm state persisted in statistically greater numbers pre-digestion (0.2-2.7 log CFU/g) and after simulated gastric digestion (0.5-4.1 log CFU/ml) compared to planktonic cells (P < 0.01). Recovery of culturable *Salmonella* was reduced after prolonged exposure to desiccation for 30 days (compared to 1-day desiccation) for biofilm (2.1-log reduction) and planktonic cells (3.3-log reduction). All cells, regardless of prior lifestyle grew within the simulated small intestinal phase, achieving final yields greater than 5 logs CFU/ml. This suggests more cells are alive but in a viable-but-non-culturable state when stressed by prolonged desiccation and low pH.

Significance: The ability to survive long-term storage as well as grow exponentially within the intestinal tract further suggests that industrial processes may influence the virulence of *Salmonella*, suggesting the need to identify different strategies for controlling *Salmonella* within dry food processing environments.

P1-101 Distribution of Antibiotic Resistant Phenotypes and Genotypes among *Salmonella* spp. Isolated from Broiler Chickens in British Columbia, Canada

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Introduction: The risk posed by antibiotic-resistant *Salmonella* in poultry products is not well understood.

Purpose: The objective of this study was to analyze the antibiotic resistance phenotype and genotype of *Salmonella* isolated from broilers in Canadian production systems.

Methods: A total of 194 *Salmonella* isolates recovered from a large commercial farm in British Columbia, Canada, were speciated and serotyped. Susceptibility to antibiotics was performed by Sensititre. Detection of virulence and antibiotic resistance genes was performed by PCR. Genetic diversity was determined by pulse-field gel electrophoresis typing (PFGE).

Results: Seventeen different serovars of *Salmonella* were identified. The most prevalent included Kentucky (29.4%), Typhimurium (15.5%), Enteritidis (13.9%), Hadar (12.4%), while 15 Heidelberg, 10 Enterica, 9 Brandenburg and 7 Thompson isolates were also recovered. Overall, resistance to ampicillin (45.7%), amoxicillin-clavulanic acid (43.2%), ceftiofur (42.6%) cefoxitim (41.6%), tetracycline (29.4) and streptomycin (21.3%) was most common. Thirty-three (57.9%) of the serovar Kentucky isolates were resistant to amoxicillin-clavulanic acid-ampicillin-cefoxcitin-ceftiofur, and two isolates were also resistant to chloramphenicol, streptomycin, sulfisoxazol and tetracycline. Genes associated with resistance to aminoglycoside (*aadA1*, *aadA2*, *strA*), β-lactam (CMY-2, SHV, TEM), tetracycline (*tetA* and *tetB*) and sulfonamide (*sul1*) were detected in all 194 isolates. The invasin (*invA*) and virulence (*spv*) genes were found in 63.4% and 7.7% of the isolates, and 10 of the 27 (37.0%) *S.* Enteriditis carried both *invA* and *spv*. PGFE typing revealed that the antibiotic resistant serovars were genetically diverse.

Significance: The data confirmed that broiler chickens are colonized by genetically diverse antibiotic resistant *Salmonella* harboring virulence determinants. The presence of such strains is highly relevant to food safety and public health.

P1-102 Comparative Evaluation of the 3M™ Molecular Detection Assay *Escherichia coli* O157 (including H7) for the Detection of *Escherichia coli* O157 in Foods

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Introduction: The 3M™ Molecular Detection Assay *E. coli* O157 (including H7) is designed for rapid and specific detection of *E. coli* O157 following 8-18 hours of enrichment. The assay uses a combination of isothermal amplification of unique DNA target sequences and bioluminescence to detect the amplified sequences. Presumptive positive results are reported in real-time while negative results are displayed upon completion of the assay.

Purpose: The purpose of this evaluation was to compare the 3M™ Molecular Detection Assay *E. coli* O157 (including H7) method to the FDA/BAM for spinach and sprouts and the USDA/FSIS-MLG method for raw ground beef.

Methods: The method comparison was conducted on three food matrices using the 3M™ test method and a reference method (FDA/BAM or USDA/FSIS-MLG). Each matrix was inoculated with a different strain of *E. coli*O157 and 20 replicates were analyzed at a fractionally positive level of 2.3-3.75 CFU/sample. Five control replicates were analyzed at 0 CFU/25g. For the new method, replicates were enriched in BPW (3M ISO formulation) for 8-18 hours at 41.5 ± 1°C. Produce enrichments (spinach 200 g, sprouts 25 g) were prepared as 1:10 dilutions and beef enrichments (325 g) as 1:4 dilutions. DNA was extracted and analyzed by the new assay, and replicates confirmed by the reference methods.

Results: No significant differences were observed between the new method and the reference methods as indicated by McNemar's X^2 (> 3.84) for the three foods. For foods compared to the FDA/BAM, X^2 = 2.01 for spinach and 1.52 for sprouts. For foods compared to the USDA/FSIS-MLG, X^2= 0.0 for raw ground beef.

Significance: For the foods evaluated, the 3M method demonstrated comparable results to the reference methods for the rapid, automated detection of *E. coli* O157.

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P1-103 A Comparative Evaluation of the 3M™ Molecular Detection Assay *Salmonella* for the Detection of *Salmonella* in a Variety of Foods

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Introduction: The 3M™ Molecular Detection Assay *Salmonella* is designed for rapid and specific detection of *Salmonella*in food, feed and food process environmental samples after 18-24 hours of enrichment. The assay uses a combination of isothermal amplification of unique DNA target sequences and bioluminescence to detect the amplified sequences. Presumptive positive results are reported in real-time while negative results are displayed upon completion of the assay.

Purpose: The purpose of this internal evaluation was to compare the 3M™ Molecular Detection Assay *Salmonella*method to the FDA/BAM for raw shrimp, bagged spinach and wet-pet food and the USDA/FSIS-MLG method for raw ground beef, cooked breaded chicken and pasteurized liquid whole egg.

Methods: The method comparison was conducted on 6 food matrices by the new method and the FDA/BAM or USDA/FSIS-MLG. Each matrix was inoculated with a different strain of *Salmonella*and 20 replicates were analyzed at one inoculum level: 0.2-2 CFU/test portion. Five control replicates were analyzed at 0 CFU/test portion. For the new method, replicates were enriched in BPW (ISO formulation) for 18-24 hours at 37 ± 1°C, DNA was extracted and analyzed by the new assay, and replicates confirmed by the reference methods.

Results: No significant differences were observed between the new method and the reference methods as indicated by McNemar's X^2 (> 3.84) for all 6 foods. For foods compared to the FDA/BAM, X^2 = 1.00 for raw shrimp, 0.41 for bagged spinach and 1.00 for wet pet food. For foods compared to the USDA/FSIS-MLG, X^2= 1.71 for raw ground beef, 0.10 for cooked breaded chicken, and 3.49 for pasteurized liquid whole egg.

Significance: For all foods evaluated, the 3M™ Molecular Detection Assay *Salmonella* demonstrated comparable results to the reference methods for the rapid, automated detection of *Salmonella*.

P1-104 Effect of Bovamine® on Prevalence and Concentration of *Escherichia coli* O157:H7 and Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) on Beef Feedlot Cattle

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Introduction: Cattle are common reservoirs for STEC without presenting any clinical symptom. *E. coli* O157:H7 along with other non-O157 strains (O26, O45, O111, O121, O103, and O145) have been declared as adulterants in ground beef and its components. Bovamine®, a combination of a lactic-acid-producing bacterium (*Lactobacillus acidophilus* NP51) and a lactic-acid-utilizing bacterium (*Propionibacterium freudenreichii* NP24) was tested as a direct-fed microbial to reduce the population of STEC in cattle.

Purpose: To evaluate the effect of Bovamine®; rumen culture feeding programs, low dose (LD), high dose (HD), and a combination of LD and HD on prevalence and concentration of *E. coli*O 157:H7 and non-O157 STEC.

Methods: Steer were randomly allotted into 10 pens per treatment with 10 head per pen with the following treatments administered daily: High dose Bovamine (10^9/head/day), Low Dose (10^7/head/day), and Low/High (10^7/head/day followed by 10^9/head/day at the last 30 days of feeding period). Fecal samples were collected at harvest. *E. coli* O157:H7 were recovered through immunomagnetic separation (IMS) followed by confirmation and enumeration using most probable number technique. Non-O157 STEC were detected using the BAX® System.

Results: The prevalence of O157 was low at 8-13%. The quantitative amount of O157 in the controls was 2.46 MPN/g, 0.14 MPN/g in the high dose, 1.04 MPN/g for the low dose, and 0.49 MPN/g in the Low/High dose. Prevalence of O26 was 61.7% in the control animals and 36.3% in the animals fed the high dose. The high dose also significantly reduced O103 from 55.6% in the control animals to 40%. Serogroup O45 was at 67.9% in the control animals and was reduced to 45% by the high-dose treatment. The prevalence of the other STECS was less than 10% with no differences detected among treatments.

Significance: Control of STEC in cattle using Bovamine may impact the prevalence of *E. coli* O157:H7 and non-O157 in meat products and have a positive result with regards to public health.

P1-105 A Longitudinal Study on *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* Isolated from Ready-to-Eat Meat Processing Facilities

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Developing Scientist Competitor

Introduction: *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* are foodborne pathogens of public health importance that present a significant food safety challenge to meat processors in the US. Knowledge gaps currently exist regarding the ecology, transmission, and persistence of these microorganisms in the food processing environment. Filling these gaps can facilitate development of methods to mitigate persistence, especially for small and very small facilities with limited resources.

Purpose: The objectives of this study were to conduct longitudinal sampling of 2 ready-to-eat (RTE) meat processing facilities to probe the ecology of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, to identify harborage sites where pathogens persist, and to assemble a set of isolates from harborage sites.

Methods: Briefly, sponge samples of environmental sites, food contact surfaces, and finished products were collected from the 2 facilities over a 6 month period. Up to 55 sites in each facility were sampled monthly with 3 sponge samples (for individual pathogens) taken from adjacent locations at each site. Samples were processed following modified versions of the USDA-FSIS Microbiology Laboratory Guidebook protocols 4.05, 5.05, and 8.07 for isolation of *S. enterica*, *E. coli*O157:H7, and *L. monocytogenes*, respectively.

Results: Five samples from Facility B (1.5%) were positive for *S. enterica*, but no sampling site produced *S. enterica* on more than 1 occasion. *L. monocytogenes* was detected in 11.2% of the samples collected from Facility A, while 14.7% of the samples from Facility B were positive for *L. monocytogenes*. Ten and 12 sites tested positive for *L. monocytogenes* on more than 1 sampling date in Facility A and Facility B, respectively. *E. coli* O157:H7 was not isolated from either facility over the course of the study, and *S. enterica* was not detected in Facility A.

Significance: These data suggest that *E. coli* O157:H7 and *S. enterica* are likely only transiently present in RTE meat processing facilities, while environmental persistence of *L. monocytogenes* in RTE meat plant harborage sites may be more common.

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P1-106 Detection of Viable *Escherichia coli* O157:H7 by Propidium Monoazide Real-Time PCR

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Introduction: *Escherichia coli* O157:H7 associated with food has caused many serious public health problems in recent years. However, only viable cells of this pathogen can cause infections, and false-positive detection caused by dead cells, can lead to unnecessary product recalls.

Purpose: The objective of this study was to develop and optimize a method that combines propidium monoazide (PMA) staining with real-time PCR to detect only viable cells of *E. coli* O157:H7. PMA is a dye that can penetrate dead cells and bind to cellular DNA, preventing its amplification via a subsequent PCR. Compared with ethidium monoazide (EMA), another DNA-binding, PMA has been reported to exert less influence on DNA amplification from viable cells.

Methods: Each of three strains of *E. coli* O157:H7 (505B, G5310 and C7927) was prepared separately and serially diluted to generate cell suspensions ranging from 10 to 10^8 CFU/ml. Dead cells were obtained by heating the suspensions at 85 °C for 35 min. Suspensions were then treated or untreated with PMA. DNA was extracted and amplified by TaqMan® real-time PCR targeting the *uidA* gene to detect only viable *E. coli* O157:H7 cells.

Results: A 5-min treatment with 50 mM PMA on ice was the most effective method to bind DNA from 10^8 CFU/ml dead cells. PMA-real-time PCR assay could detect as low as 10^2 CFU/ml viable *E. coli* O157:H7 in live-cell suspensions. This assay could detect 10^6 CFU/ml of viable cells when dead cells ranging from 10 to 10^6CFU/ml were present. Studies detecting lower concentrations of viable cells in the presence of dead cells and in food are ongoing.

Significance: In conclusion, the PMA-real-time PCR assay can effectively prevent amplification of DNA in dead cells of *E. coli* O157:H7 and differentiate viable from dead cells.

P1-107 Norovirus Transfer between Hands and Fresh Produce

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Developing Scientist Competitor

Introduction: Norovirus is a common cause of gastroenteritis worldwide. Many outbreaks in the US have been attributed to transmission through fresh produce and multi-ingredient foods, which often require significant handling immediately prior to consumption.

Purpose: To evaluate the effect of gloved and ungloved hands on norovirus transfer during fresh produce preparation.

Methods: The palm and fingers of bare or gloved hands (polyvinyl, vinyl or nitrile) were inoculated with 0.1 ml (approximately 6 log PFU/hand) murine norovirus (MNV-1) and allowed to dry. Freshly chopped Romaine lettuce was handled by the contaminated hands, and then sampled to determine the transfer coefficient. MNV-1 transfer was also measured after volunteers handled chopped contaminated lettuce with uncontaminated bare or gloved hands. Hands and gloves were sampled for transferred virus by the glove juice method, and recovered MNV-1 quantified by plaque assay.

Results: Transfer of MNV-1 to Romaine lettuce was greatest ($P < 0.05$) from vinyl gloves (ranging from 4.8 to 45%). In comparison, transfer coefficient of MNV-1 from bare hands or nitrile gloves to lettuce ranged from 0.1 to 5.1% and 0.02 to 8.5%, respectively. The transfer coefficient from lettuce was less variable (ranging from 0.22 and 11%), independent of glove type.

Significance: This study examined virus transfer during simulated tasks involving fresh produce preparation, and indicates norovirus transfer coefficients are affected by the surfaces involved.

P1-108 Norovirus Transfer during Chopping of Contaminated Romaine Lettuce

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Introduction: Human noroviruses are the leading cause of outbreaks of nonbacterial gastroenteritis in the U.S., and are often implicated in outbreaks associated with ready-to-eat foods, such as salads. Such foods may be extensively handled, and norovirus may be transferred readily between infected food handlers and food surfaces.

Purpose: To investigate the transfer of noroviruses between lettuce, cutting board and knife used in fresh food preparation.

Methods: Fresh Romaine lettuce was cut into 25 cm^2 pieces and inoculated with 25 µl MNV-1 (approx. 7 log PFU/leaf). After 10 min drying in a biosafety cabinet, 5-g piles of inoculated leaves were cut with a sterile stainless steel knife on a sterile polyvinyl cutting board. Sampling sponges soaked in Dulbecco's Modified Eagle's Media (DMEM) were used to swab blade and board surfaces for MNV-1 transferred from lettuce. In some cases, fresh, uninoculated Romaine lettuce was chopped using the contaminated knife and board, and MNV-1 that was transferred to the chopped lettuce was recovered by stomaching in DMEM and enumerated by plaque assay.

Results: MNV-1 transfer was lower from contaminated lettuce to cutting board ($P < 0.05$; range from 0.6 to 6.2%) than that from a contaminated cutting board to lettuce (1.6 to 91%). This same trend was observed between a stainless steel knife and lettuce. The transfer coefficient of MNV-1 was lower when contaminated lettuce was chopped on a wood board ($P < 0.05$; range from 0.1 to 2.5%) compared to a polyvinyl cutting board (0.6 to 6.2%).

Significance: Norovirus transfers readily between surfaces common in the preparation of fresh produce preparation, but the transfer coefficient appears to be dependent on the surface type. This research provides the scientific basis for the development of a risk assessment model for norovirus transfer during the fresh food preparation.

P1-109 Genotypic and Cytotoxicity Analysis of Non-O157 Shiga Toxin-producing *Escherichia coli* Isolates from Humans, Animals and Food

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli*(STEC) have become increasingly important to public health and food safety. Better understanding of non-O157 STEC is important for development of measures.

Purpose: To analyze the diversity of virulence factors among non-O157 STEC isolates from human, animal and food isolates.

Methods: A total of 311 STEC isolates (73 human, 139 animal and 99 food) were examined for the Shiga toxin gene (*stx*) variants, intimin gene (*eae*) variants and enterohaemolysin gene (*hlyA*) using PCR and PCR-RFLP. They were also classified into seropathotypes based on serotypes. Vero

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cytotoxicity of selected isolates from different sources was also determined.

Results: Most human and animal isolates belonged to seropathotype B, whereas food isolates were mainly seropathotypes C, D and E. Virulence gene profiles differed significantly among the isolates. Most human and animal isolates were positive for *stx1a* (76% and 97%), *eae* (82% and 92%) and *hlyA* (81% and 81%), respectively. The virulence gene distribution in food isolates was *stx1a* (27%), *stx2a* (35%), *stx2dact* (36%), *eae* (1%), *hlyA*(45%) and other minor virulence factors. Food isolates had a greater variety of virulence factor combinations (20) compared to human (11) and animal isolates (10). As expected, the human isolates exhibited the greatest cell cytotoxicity, whereas cytotoxicity varied among the food isolates.

Significance: STEC isolates from food significantly differed from human isolates in serotypes, virulence factors and vero cell cytotoxicity. Some food isolates, however, belonged to important serotypes, carried important virulence factors and exhibited elevated cell cytotoxicity, thereby have the potential to cause severe diseases in humans.

P1-110 Molecular and Phenotypic Characterization of *Salmonella enterica* Serotypes Typhimurium and 4,5,12:i:- in Thailand

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Introduction: *Salmonella enterica*is one of the most common causes of bacterial foodborne diseases worldwide. In Thailand, the reported salmonellosis cases are approximately 3,000 per year with serotypes Typhimurium and 4,5,12:i:- (related monophasic serotype lacking phase 2 flagella antigen) ranking among the top ten causative agents. Over the last decade, both serotypes have been characterized from various sources in Thailand and believed to be relative clones involving in multidrug resistance among Thai patients.

Purpose: The purpose of this study is to provide a better understanding of the emergence of Thai *Salmonella*serotype 4,5,12:i:- and its characteristics in comparison to serotype Typhimurium.

Methods: At least 138 of *Salmonella* isolates were provided by the National *Salmonella* and *Shigella* Center (WHO Thailand), among which 68 isolates belong to *S.* Typhimurium serotype, and the other 70 isolates belong to serotype 4,5,12:i:- isolated from various sources (e.g., farms, foods, and patients) in Thailand. These isolates were characterized using different molecular subtyping methods (e.g., polymerase chain reaction [PCR] and pulsed-field gel electrophoresis [PFGE]), and antibiotic resistance pattern (AbR).

Results: PCR results showed that Thai *S.* 4,5,12:i:- isolates (n = 60) contain a unique deletion pattern which is different from Spanish clones at the 3' end of Cluster V region. In addition, most of Thai *S.* Typhimurium isolates (n = 61) have a consistent gene presence/absence pattern with previously characterized Spanish and US Typhimurium isolates. Concurrently, antibiotic resistance study showed that 50% (34/68) of *S.* Typhimurium and 91.43% (64/70) of *S.* 4,5,12:i:- are multi-drug resistant (i.e., resistant to three or more antimicrobial agents, particularly ampicillin, cefotaxime, tetracycline, and streptomycin).

Significance: The findings from this study suggest that Thai *S.* 4,5,12:i:- strains might have distinctly emerged from *S.* Typhimurium strains, possibly those with multidrug resistant capabilities.

P1-111 Development of BAX® System Real-Time PCR Assay for *Shigella*

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Introduction: *Shigella* is the third most common pathogen transmitted through foods (higher than *E. coli* O157:H7). The infective dose of *Shigella* is as low as 10 cells, and there are an estimated 150 million illnesses and 600,000 deaths caused annually by this organism. In order to meet the urgent need for the detection of *Shigella*in foods, a Scorpion™ probe-based real-time PCR assay and an accompanying culture confirmation method were developed.

Purpose: The objectives of this study were to develop this BAX® System real-time PCR assay for detecting *Shigella* in a variety of food samples and evaluate *Shigella* selective media for confirmation, isolation and further characterization of*Shigella*species.

Methods: After enrichment, lysis was performed according to the standard BAX® System method and a full process was run in the Q7 instrument. Inclusivity, exclusivity and sensitivity were tested in 123 *Shigella* strains, 71 closely related enteroinvasive *E. coli* (EIEC) strains and 81 non-*Shigella*/EIEC strains, and PCR tablet lot-to-lot comparison was also performed. Both commercial and non-commercial *Shigella*culture media were evaluated with the inclusivity and exclusivity panel strains.

Results: The BAX® System assay followed by *Shigella* selective agar confirmation method demonstrated 100% inclusivity for the 123 *Shigella* strains tested and 100% exclusivity for the 152 non-*Shigella*strains tested. The Real Time PCR assay was sensitive enough to detect 50 CFU/ml of the target pathogen both in pure culture and food samples. Consistent results were obtained by the lot-to-lot PCR assay comparison.

Significance: This developmental Real-Time PCR assay can detect *Shigella* species at levels as low as 50 CFU/ml. Assisted by a culture confirmation method, which helps distinguish *Shigella* from EIEC strains, this protocol provides a useful tool for food companies/industries to rapidly detect *Shigella* species in foods.

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P1-112 Validation of BAX® System Real-Time PCR Assay for Detection of *Shigella* in Foods

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Introduction: A Scorpion™ probe-based real-time PCR assay and accompanying culture confirmation method were developed to detect *Shigella* species in foods. The specificity of the assay was demonstrated with a diverse range of *Shigella*species, related organisms, and unrelated genera. The assay was sensitive enough to detect less than 100 CFU/ml of the target pathogen in both pure broth culture and food samples.

Purpose: The objective of this study was to evaluate the BAX® System assay for detecting *Shigella* in foods using an enrichment in standard *Shigella* enrichment broth. Sample enrichment in BAX® System MP media was also evaluated to allow for testing of *Shigella*, *Salmonella* and *E. coli* O157:H7 from a single enrichment.

Methods: Various foods including raw and ready-to-eat meats, jelly and produce were spiked with *Shigella* species at levels likely to obtain fractional positives. Samples were enriched in either standard *Shigella* broth (as described in FDA-BAM Chapter 6, ISO21567 and new China National Standard GB4789.5) or BAX® System MP media for 8 – 20 hours, then prepared and processed according to the standard BAX®System protocol. All samples were confirmed with the appropriate reference culture method.

Results: In foods with low background microflora, such as ready-to-eat meat and jelly, the BAX® System method detected *Shigella* in 8-20 hours with 100% sensitivity and specificity regardless of enrichment method. Chi-square analysis indicates that the test method and the reference methods returned statistically equivalent results. For the remaining sample types, only slight differences were found between samples enriched

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with standard media and BAX® System MP media. Chi-square analysis indicates that these differences are not statistically significant, demonstrating that BAX® System MP media performs comparably to *Shigella* broth for the enrichment of *Shigella* in these food types.

Significance: Because this study demonstrates that the BAX® System method is comparable to the reference method and that BAX® System MP media can be used to enrich samples for *Shigella* testing, these results suggest that it is possible to enrich certain samples for *Shigella*, *Salmonella* and *E. coli* O157:H7 testing from a single 8 – 20 hour enrichment in BAX® System MP media, saving time, effort and expense for customers. However, it remains a challenge to isolate *Shigella* spp. from certain foods which has high background microflora such as raw pork using conventional culture media.

P1-113 Comparison of Phage-based Magnetoelastic Biosensors with TaqMan-based Quantitative Real-Time PCR for the Detection of *Salmonella* Typhimurium Directly Grown on Spinach Leaves

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Introduction: Increasing outbreaks of *Salmonella* on fresh produce over the past decade point to the need for an inexpensive, simple, fast, and sensitive detection method. A phage-based magnetoelastic (ME) biosensor has been developed as a practical on-site detection method.

Purpose: In order to evaluate the ME biosensor method, the ME biosensor method was compared with a TaqMan-based quantitative real-time PCR (qPCR) method.

Methods: Topological characteristics of adaxial and abaxial surfaces of spinach leaves were observed using scanning electron microscopy (SEM). Standard curves, correlations, and limits of detection (LOD) for the ME biosensor and qPCR methods were determined by inoculating *S.* Typhimurium suspensions (ranging from 1 to 8 log CFU/spinach) on the surface of spinach leaves. For the comparison of the detection methods, the *S.* Typhimurium suspension (3 log CFU) was directly inoculated and grown on 25 adaxial surfaces of spinach leaves. After 24 h, both methods were performed to detect *S.* Typhimurium on the spinach leaves.

Results: Although SEM microscopic images showed the topological difference of adaxial and abaxial surfaces, the distribution of *S.* Typhimurium was nearly similar on both sides. The LOD for the ME biosensor and qPCR were 3 ± 0.19 and 2 ± 0.11 log CFU/spinach, respectively. After 24 h, *S.* Typhimurium was positively detected by both methods and the quantified concentrations were 5.78 ± 0.79 and 5.89 ± 0.26 log CFU/spinach for the ME biosensor and the qPCR method, respectively. SEM was used to confirm the growth of *S.* Typhimurium on the spinach surfaces and the binding of *S.* Typhimurium on the measurement sensors.

Significance: This study demonstrates that the ME biosensor method was robust and competitive with qPCR and has great potential to serve as an on-site and in-field detection method, in order to improve the safety of fresh produce.

P1-114 Mechanisms of Inactivation Affect the Relationship between Viral Attachment and Infectivity

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Introduction: Studying norovirus infectivity and persistence in the environment and in food processing is limited due to the inability to propagate norovirus in cell culture and in animal models. Varying processing treatments have differing mechanisms of inactivation which have the potential to affect attachment properties.

Purpose: This study aims to determine if viral attachment through an ELISA is a means to determine infectivity, using heat, high pressure, ozone and UV-treated murine norovirus (MNV) by correlating ELISA optical density results to plaque assay results for infectivity.

Methods: ELISA plates were coated with porcine gastric mucin (PGM) and untreated and heat-, HPP-, ozone- and UV-treated MNV was added followed by monoclonal anti-MNV antibody. Anti-IgG antibodies were detected by horseradish peroxidase conjugated goat anti-mouse IgG antibody. The average OD_{405} of MNV-containing wells were divided by negative control wells and expressed as the 'P/N ratio'; values ≥ 2 were considered positive. Infectivity of MNV following treatments was determined using the plaque assay. Capsid integrity was determined by RNase treatment and subsequent RT-PCR. Positive control wells were coated with virus sample and negative controls included wells without antibody, virus, or PGM coating.

Results: Heat-treated viral attachment decreased significantly with decreasing viral infectivity. Heat treated the P/N ratios were 1.86 ± 0.28 and 1.83 ± 0.03, respectively, at 80 and 100 °C, and attachment was not considered to be positive. This correlated MNV inactivated beyond the limit of detection and the MNV capsid was not intact as shown by RNase treatment. HPP-, ozone- and UV-treatment of MNV had no significant difference in attachment from control untreated virus, and the P/N Ratio was ≥ 2 for all treatments where inactivation was beyond the limit of detection. The capsids of HPP-, ozone- and UV-inactivated MNV remained intact as determined by RNase treatment.

Significance: The relationship between attachment and infectivity varied for the different food processing treatments, whereby, heat-inactivated MNV was the only treatment where this relationship existed. This observation suggests that partial destruction of the viral capsid is necessary to observe any changes in viral attachment to host cell receptors.

P1-115 Optimization of Extrusion for the Inactivation of *Enterococcus faecium* NRRL-B2354 Using a Response Surface for Moisture and Temperature

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Introduction: Outbreaks of salmonellosis and multiple recalls of low moisture foods including extruded products highlight the need for industry to validate their extrusion processes to ensure the destruction of pathogenic microorganisms. The use of surrogate organisms allows for studies to be conducted with safer organisms than pathogens. *Enterococcus faecium* NRRL B-2354 has been previously reported as an appropriate surrogate for *Salmonella* for the validation of thermal processes in almonds and was used in this study to validate the extrusion process for inactivation of *Salmonella enterica*.

Purpose: Response surface methodology was employed to study the effect of moisture and temperature on inactivation by extrusion of *Enterococcus faecium* NRRL B-2354 in a carbohydrate-protein mix.

Methods: A balanced carbohydrate-protein mix was formulated to different combinations of moisture contents ranging from 25.8 to 31.1% and each was inoculated with a pure culture of *Enterococcus faecium* NRRL-B2354 to a final level of 5 logs or above. Each mix of varying moistures was then extruded in a pilot scale extruder at different temperatures (set points ranging from 65°C to 85°C). At each combination of moisture and temperature the extruder was allowed to equilibrate for 10 minutes before sample collection. Samples were collected in sterile bags, cooled in dry ice, and stored at 4°C prior to analysis. *E. faecium*was enumerated using TSA and mEnterococcus media followed by incubation at 35°C for

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48 hours. Each extrusion was repeated twice, with the central point of the design being repeated four times. From each extrusion, 3 subsamples were collected for microbial counts and moisture determination.

Results: Based on the response surface analysis of the results, temperature and moisture combinations above 77°C and 28% moisture would completely eliminate *E. faecium*, with the main factor being temperature. Other temperature and moisture combinations needed to achieve specific log reductions were plotted in a three-dimensional response surface graph that can be referenced by industry. Decreasing moisture and temperature combinations resulted in higher survivability of the microorganism.

Significance: The response surface model showed a very good coefficient of correlation (above 0.9) with temperature having a significant effect on the survivability of *E. faecium* NRRL-B-2354 during extrusion. The response surface model gives industry a tool to better understand the effect of moisture and temperature on the inactivation of heat resistant microorganisms in their processes.

P1-116 Comparison of Ultraviolet Light against Log and Stationary Phase *Listeria monocytogenes*

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Developing Scientist Competitor

Introduction: The ubiquitous nature of *Listeria monocytogenes* and its ability to grow at refrigerated temperature has made *L. monocytogenes* a significant threat to the safety of ready-to-eat (RTE) meat products. Contamination by *L. monocytogenes* in RTE meat primarily occurs during slicing and packaging as a post process re-contamination. A non-thermal, post-process decontamination method was applied to study the efficacy of UV light against *L. monocytogenes*at different exposure times and intensities.

Purpose: To evaluate the efficacy of ultraviolet light against log and stationary phases of *Listeria monocytogenes* in growth media.

Methods: *Listeria monocytogenes* serotype 4a was cultured in Brain Heart Infusion (BHI) broth at 37°C. Cells were harvested during their log and stationary phase and 1.5 ml of the cell suspension (3 mm in depth) was taken in a 3 cm petriplate, subjected to UV radiation at low (3 – 4 mW/cm^2) and high (7-8 mW/cm^2) intensity for 0, 10, 30,50,70,90 and 110 s. Cells were recovered on Modified Oxford agar (MOX) and data was analyzed using ANOVA to find significant differences between the means at $P < 0.05$.

Results: Overall, populations of *L. monocytogenes* were significantly reduced ($P < 0.05$) after 10 and 30 s of exposure and further significant ($P < 0.05$) reductions were observed at 50, 70, 90, and 110 s when compared to the 10 and 30 s exposure times irrespective of the growth phase and UV light intensities. Irrespective of the UV light intensity and exposure times, significantly higher ($P < 0.05$) reductions were observed in the log phase cells as compared to the stationary phase cells.

Significance: Significant reduction in the log phase of *L. monocytogenes* populations as compared to the stationary phase are of importance for processors as it provides information that is helpful to design an effective UV light intervention system.

P1-117 Characterization of *agr* Groups in *Staphylococcus aureus* Strains and Association with Classical Enterotoxins Genes, Coagulase and Thermonuclease

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Introduction: *Staphylococcus aureus* virulence is mainly determined by the production of enterotoxins (SE), as well as cell surface-associated proteins. The accessory gene regulatory (*agr*) operon plays an essential role in regulating this virulence. In this context, *S. aureus* strains can be grouped into four interference profiles according to allelic variations in *agr*.

Purpose: Characterize a population of *S. aureus* strains based on *agr* polymorphism, associating to the presence of classical SE genes (*sea*, *seb*, *sec*, *sed*, and *see*), and production of coagulase and thermonuclease enzymes.

Methods: Eighty-three *S. aureus* isolates obtained from milk and cheese were subjected to *agr* group-specific multiplex PCR. Additionally, classical SE genes were also detected by PCR. The production of classical SE was determined by using a commercial enzyme-linked immunosorbent assay kit. Finally, the strains capabilities of producing coagulase and thermonuclease were evaluated by biochemical methods.

Results: Typing of the *agr* operon revealed the predominance of *agr* group III (97.6%), followed by *agr* group I (68.7%) and *agr* group II (56.6%). Only 2 strains were found to harbor *agr* group IV (2.4%). The most common association between *agr* polymorphisms found among the isolates in this study was *agr* groups I and III. The *agr* III genotype strains were found to be more related to positive results in both coagulase (76.5%) and thermonuclease (79.0%). The *agr* I genotype strains were found to possess higher capabilities of producing SE (22.8%) and more likely to harbor *see* (43.9%), *sea* (40.4%) or *sec* (38.6%) genes. Among all tested SE genes, *sea* was found to be the most prevalent, being more frequently associated to *agr* group II (46.8%) strains.

Significance: The obtained results can be helpful in the context of the current understanding of the contribution of the *agr* operon to the virulence of *S. aureus* strains isolated from milk and cheese.

P1-118 Influence of Water Mobility on the Survival of *Salmonella* spp. in Low-moisture Whey Protein Powder at 80°C

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Introduction: *Salmonella* can survive in dry food for long periods of time. Reduced microbial inactivation in low moisture food during heating is believed to be due to the interaction of cells and water, and is often thought to be related to water activity (a_w). Water mobility is a measure of the ability of water molecules to translocate in the food. Little is known about the role of water mobility in influencing the survival of *Salmonella*in low moisture foods.

Purpose: The aim of this study is to determine the influence of water mobility on the survival of *Salmonella*in low moisture whey protein powder held at 80°C.

Methods: Whey protein powder (95% protein) of differing water mobilities was produced by pH adjustment (2, 5, and 7), heat denaturation and equilibration to various a_w levels between 0.18 and 0.52. Powders were inoculated with a four-strain cocktail of *Salmonella*, vacuum-sealed and immersed in a circulating water bath at 80 °C for 48 hr. Cells were recovered at various times on nonselective differential media. Microbial survival data were analyzed using ANOVA.

Results: Water activity significantly influences the heat inactivation of *Salmonella* ($P < 0.0001$). Treatment after 4 hours showed average log reduction values of 2.7, 4.6, 5.0, 5.2 and 5.1 at a_w levels of 0.18, 0.29, 0.36, 0.42 and 0.52, respectively. Water mobility had a significant effect on microbial death ($P = 0.030$) at higher a_w levels (0.36 to 0.52), with average log reductions of 4.8 and 5.4 at lower and higher mobilities, respectively. Water mobility did not significantly influence inactivation in the a_w range of 0.18 to 0.29 ($P = 0.598$).

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Significance: Water mobility influences the survival of *Salmonella* spp. in low moisture protein powder at a_w levels between 0.36 and 0.52 during treatment at 80 °C.

P1-119 Comparative Survival of Shiga-toxin Producing *Escherichia coli* in Ground Beef with Different Fat Levels under Conditions Resembling Refrigeration, Partial Cooking and Digestion

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Developing Scientist Competitor

Introduction: In the fall of 2011, the USDA declared six non-O157 Shiga toxin-producing serogroups of *Escherichia coli*(STEC) as legal adulterants in non-intact raw ground beef due to the threat that these organisms present to public health. The need to understand the behavior of foodborne non-O157 STEC exposed to the human gastric environment is crucial to understanding their role in public health.

Purpose: This study compared survival of non-O157 STEC (6 strains from serogroups O26, O45, O103, O111, O121 and O145) and the known heat-acid-tolerant O157:H7 strain ATCC 43895 in 15% and 27% fat ground beef during sequential refrigeration, partial cooking, and incubation in a simulated gastric environment.

Methods: Inoculated ground beef (25 g) was refrigerated (4°C) for 16-17 h and then heated 30 min at 54.4°C to mimic partial cooking. After cooling in ice 10 min, the inoculated beef was combined with pre-warmed (37 °C) Synthetic Gastric Fluid (pH 2.0; 70 ml) and held at 37°C. Surviving cells were enumerated before and after heating and after 0.5, 1, 2, 3, 4, and 6 h of incubation in SGF. Overall changes in population (D-log CFU/g) were calculated for each strain separately at the 6-h sampling time, relative to the level before heating.

Results: STEC populations decreased by 1.5 – 3.0 log CFU/g or by 0.8 – 3.5 log CFU/g during cooking in 15% and 27% fat ground beef, respectively, and by 1.4 – 5.7 log CFU/g during subsequent 6-h incubation in SGF in both 15% and 27% fat ground beef. In 27% fat ground beef there were very significant ($P < 0.01$) inter-strain differences in survival. One strain each from serogroups O26, O103, and O111, and the O157:H7 reference strain all survived significantly better than other strains tested. There was no statistical difference in D-log CFU/g when results for 15% and 27% fat ground beef were compared, except for one strain of serogroup O26 which exhibited significantly lower D-log CFU/g values in 15% ground beef.

Significance: Several non-O157 STEC strains were shown to survive as well as O157:H7 in ground beef when exposed to sequential refrigeration, partial cooking, and synthetic gastric fluid. Fat level, 15% or 27%, did not affect STEC survival.

P1-120 Validation of Lactic Acid Dip and Spray in Reducing *Escherichia coli* O157:H7, *Salmonella*, and Non-O157 Shiga-toxigenic *Escherichia coli* (STEC) on Beef Trim and Ground Beef

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Introduction: Organic acids are frequently applied to beef carcass tissues as an antimicrobial intervention to reduce *E. coli* O157:H7, non-O157 STEC, *Salmonella*and other pathogenic populations that may be present.

Purpose: The objective of this research was to compare the effectiveness of two different application methods (dip vs. spray) of 4.4% lactic acid on reducing *E. coli* O157:H7, non-O157:H7 STEC and *Salmonella* on inoculated beef trim and ground beef.

Methods: Beef trim inoculated with *E. coli* O157:H7, non –O157 STEC or *Salmonella* (10^5 to 10^6CFU/g) at separate times was subjected to four different treatments: 4.4% lactic acid dip (LD), 4.4% lactic acid spray (LS), water dip (WD), water spray (WS), plus an inoculated, untreated control (CTL). Intervention effectiveness on pathogen reduction was measured at the following processing points: 1 h and 20 h after treatment (trim), and 1 h, 24 h, 72 h and 7 days after grinding (ground beef).

Results: The LD treatment reduced all pathogens significantly ($P < 0.05$) on both beef trim and ground beef. The LD reduced *E. coli* O157:H7 by 0.91 to 1.41 log cycles on beef trim and ground beef, STEC by 0.48 to 0.82 log cycles, and*Salmonella* by 0.51 to 0.81 log cycles. No other treatment, including the LS treatment, significantly reduced ($P > 0.05$) any pathogen from the CTL.

Significance: These data indicate that a lactic acid dip treatment was more effective at reducing *E. coli* O157:H7, *Salmonella* and non-O157 STEC when compared to a conventional lactic acid spray treatment. Further research is needed to determine if factors such as temperature, acid type, acid concentration and length of exposure impact the effectiveness of a dip treatment on these pathogens.

P1-121 Identification of Variable Regions within Genomes of Shiga Toxin Prophage from *Escherichia coli* O157:H7

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Introduction: Shiga toxin-producing *E. coli* O157:H7 have been implicated in foodborne outbreaks linked to vehicles including fresh produce and ground beef. They are especially dangerous pathogens due to their low infectious dose. The*E. coli* O157:H7 Sakai genome encodes genes for Shiga toxin 2 (stx_2) within a prophage designated Sp5. Due to the modular nature of phage genomes, there is a high level of sequence polymorphism between these phage isolated from different strains of *E. coli*O157:H7 which needs to be better defined.

Purpose: Identification of genetic differences between stx_2-encoding phage may help improve DNA sequence-based subtyping approaches for identifying transmission routes of *E. coli* O157:H7 during outbreaks.

Methods: The stx_2-encoding phage from twenty clinical isolates, including the prototypical strains EDL933 and Sakai were isolated after ciprofloxacin induction and sequenced by 454 technology.

Results: Comparison of phage sequences identified most sequence variation in genes annotated previously as the encoding cII and cIII protein, exonuclease, recombination protein Bet, host-nuclease inhibition protein Gam, Kil, replication protein O, antirepressor, DNA binding protein, antiterminator Q and C4 Zn-finger protein. Moreover, we observed that the presence and position of an insertion sequence (IS*629*) element varied. Additionally, the sequenced phages were more similar to Sp5 from strain Sakai than the stx_2-encoding phage from EDL933. We also observed differences in the expression of Stx_2between strains.

Significance: The regions identified in this study might be useful as markers to increase the discriminatory power of multilocus sequence typing protocols for *E. coli* O157:H7 and sequence analysis may provide insights into the responsible mechanism(s).

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P1-122 Development of Predictive Models for Foodborne Disease Outbreak Due to Climate Change in Korea

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Introduction: Climate change may have direct and indirect impact on the food safety at various stages of the food chain in Korea. Especially, it is expected to cause an increase in the foodborne disease outbreaks (FBDOs). Therefore their corresponding studies have been actively conducted in many country. However accurate prediction of FBDOs requires considering the differences of pattern in FBDOs between countries and regions and developing a model by appropriately utilizing the major variables of climate change.

Purpose: Therefore, in this study, to identify the impact of climate change on FBDOs in Korea, predictive models were being developed using climate variables in consideration of regional differences of Korea. Also, future trends of FBDOs due to climate change in Korea were predicted by the mathematical model combining climate change scenario.

Methods: We analyzed datasets on FBDOs together with 18 climate variables in Korea during the period 2002-2011, and developed the Poisson GLM, ARMA, and Auto-Reg models. Notified FBDOs in 2011 were used to test the predictive ability of the models. Parameter estimation, goodness-of-fit and predictive ability (AIC, BIC, and MSE) of the models were compared. For the future trends of FBDOs, the climate change scenario produced in KMA (Korean Meterological Administration) based on the SERS A1B emission scenarios were used.

Results: The results suggested that the Auto-Reg and ARMA models produced the highest predictive ability; however, Poisson GLM and Poisson AutoRegressive models produced significantly large MSEs, indicating relatively lower predictive abilities than the other models. Though the high relationship was shown between meteorological variables, average temperature was all positively correlated with FBDOs in three climatic regions classified by temperature and rainfall patterns in Korea. The impact of climate change will exacerbate incidences of foodborne disease in Korea, so until 2040 about 1.3 (outbreaks) and 1.5 (cases) fold increase with regional and seasonal variation.

Significance: This is the first study to examine the association between climate variability and FBDOs using different predictive models considered regional variations in Korea. The predictive models play an essential tool for developing food safety programs and climate change adaptation in Korea.

P1-123 A Review of Nosocomial *Salmonella* Outbreaks: Effective Infection Control Interventions

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Introduction: Hospitals can be viewed as confined communities composed of individuals who are immunocompromised for a variety of reasons making them more susceptible to infectious disease. *Salmonella*can be introduced into the hospital setting by admissions, visitors, health care workers (HCWs) or food sources.

Purpose: The purpose of this study was to review documented outbreaks of enteric illness associated with nosocomial salmonellosis to identify mode of transmission, morbidity and mortality patterns and recommendations for control and prevention.

Methods: Searches of electronic databases, public health publications, and federal, state and provincial public health websites were completed.

Results: Computer-aided searches of literature databases and systematic searches of government websites identified 52 relevant outbreak reports. The most commonly reported routes of transmission were food 31/52 (59.6%) and person-to-person 7/52 (13.5%), while 14/52 (26.9%) reported "other" or "unknown." Actions taken during the outbreak to control transmission included improvements to infection control practices (41.8% of all actions); isolation or cohorting patients, improving hand hygiene practices and enhancing cleaning and disinfection in patient care areas, and; improvements in food handling (24.4%) through reviewing food preparation practices, enhanced cleaning and sanitation of the kitchen and control of food temperatures. Investigators made recommendations retrospectively in outbreak reports to provide direction to health centers in an attempt to prevent outbreaks in the future. These recommendations are not statistically supported because unlike intervention strategies in other sectors, it has not been possible to conduct trials to evaluate the effectiveness of one recommendation compared to another.

Significance: Although many recommendations were similar to control actions, centering on improving infection control practices important to prevent secondary transmission of salmonellosis, this study suggests that more emphasis should be placed on improving food handling practices, such as training food workers, monitoring food temperatures, and not using raw foods of animal origin, because almost 60% of the outbreaks were foodborne.

P1-124 A Restaurant Food Handler Knowledge Assessment in a French-speaking Canton of Switzerland

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Developing Scientist Competitor

Introduction: There have been no published studies of restaurant food handler food safety knowledge in Switzerland. Identifying food safety knowledge gaps may help target educational messages for restaurant food handlers to promote optimal behavior.

Purpose: To identify gaps in food safety knowledge among restaurant food handlers in Neuchâtel, Switzerland.

Methods: Between November 2010 and February 2011, an oral 54-question survey that included 46 knowledge questions was administered in French and English to 100 food handlers in 100 randomly sampled Neuchâtel restaurants. Restaurants were selected if the local food safety officers believed the restaurant was likely to participate.

Results: The mean age of food handlers was 43 years (range 18 to 65 years). Sixty-seven percent were males and 11% had attained no higher than high school education. The mean knowledge score was 32.7/46 (71%). Twenty-seven percent of the food handlers knew that it is a safe food practice to transfer a large pot of hot soup into small containers and place those small containers in a refrigerator to cool. Only 7% knew that raw beef may cause hospitalization or even death. None of the food handlers knew the correct temperatures for cooking chicken and holding potentially hazardous hot foods, the time and temperature recommendations for holding potentially hazardous cold foods (such as mayonnaise) without temperature control and the range of temperatures for pathogen growth.

Significance: We observed substantial food safety knowledge gaps among restaurant food handlers in Neuchâtel, Switzerland, that may place restaurant consumers at risk for food poisoning. Data from this study demonstrate that time and temperature issues and understanding the consequences of consuming incompletely cooked meat and poultry should be priorities for food handler education.

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P1-125 Stakeholder Engagement in an Interactive Scoping Study of the Role of Wildlife in the Transmission of Pathogenic Bacteria and AMR to the Food Chain

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Introduction: Synthesis research uses systematic and transparent methodologies such as scoping studies, systematic reviews and meta-analysis, to summarize available evidence in support of decision making. To improve the utility of our research we developed a protocol for conducting interactive synthesis research to improve question and end product development.

Purpose: This research focused on testing and validating a protocol for stakeholder advisory group (AG) engagement within our interactive synthesis research framework. The topic of the interactive scoping study is the role of wildlife in the transmission of pathogenic bacteria and antimicrobial resistance (AMR) to the food chain. After identification, the AG was engaged in development of the question, scope and products for this project.

Methods: By personal invitation, an AG of 11 experts from wildlife biology, microbiology, food safety and epidemiology was established. A survey was administered to solicit insight on the scope and framing of the study questions.

Results: We had a positive response rate of 100% to the AG committee invitations. The survey helped validate and expand the project scope and rank the importance of specific questions for research and decision making. For example, the research importance of the role of wildlife in the transmission of bacterial pathogens and AMR rated 4.7 (SD 0.47), compared to its policy relevance 4.2 (SD 0.79). Questionnaire results were summarized and consensus was reached on the final scope of the project and targeted end users.

Significance: Our experience with interactive synthesis research shows that the information and discussion spawned by the AG is invaluable for framing the right questions and addressing an appropriate scope during synthesis research. The AG represents a group of potential end users whose feedback helps improve the utility of synthesis products. The interactive aspects of this scoping study on the role of wildlife in the transmission of pathogenic bacteria and AMR to the food chain are key to improving the use of research in policy and decision making.

P1-126 Foodborne and Waterborne Diseases in Four World Regions Using Informal Reporting Methods, 2009-2010

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Introduction: Diarrheal diseases, which are associated with food and water contamination, kill approximately 2.2 million people per year worldwide. In some regions, formal reporting methods, which use comprehensive disease surveillance systems, are not available or are very limited for foodborne and waterborne diseases. Informal reporting methods, which are publically available via online sources, provide a means for supplementing formal reporting with real-time information on outbreaks.

Purpose: The goal of this study was to determine whether informal reporting methods can be used in collaboration with formal reports (information primarily from governmental sources) to better understand the regional trends and burden of foodborne and waterborne diseases.

Methods: The website, Safe Food International Regional News, was developed to gather informal reports on foodborne and waterborne diseases in six world regions. Articles were sorted by region and year (2009 and 2010). Four of the six regions met inclusion requirements for analysis. Data was extracted from each article for analysis. Statistical tests were used to determine if there were significant differences between regions and years in case fatality and cumulative incidence rates using the following factors: disease-causing agents, pathways, start month/season, and information source. In addition, results of informal reports for cholera were compared to the WHO's formal cholera database for each region.

Results: Almost 400 disease events between 2009 and 2010 were analyzed. There were statistically significant differences in case fatality rates between the world regions - with Africa having the highest rate. Informal reporting results for cholera were compared to official WHO results for cholera. In many regions, informal reports closely matched the formal reports in terms of the virulence and pathogenicity of cholera. Additionally, some informal reports added valuable information on outbreaks not captured via formally reporting.

Significance: Formal reporting systems vary globally. While comprehensive formal reporting systems provide the best information for making public health decisions, where such systems do not exist, informal reporting can address knowledge gaps.

P1-127 Estimating the Relative Risk of Raw Dairy Consumption in the State of California Using Online Resources

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Introduction: The controversy over raw dairy consumption continues to incite spirited debate between advocates of raw dairy and public health authorities. The internet has provided a convenient outlet for any and all opinions, but little in the way of easily accessible and interpretable figures of any excess risk associated with raw dairy consumption is to be found online.

Purpose: We have attempted to compute an accurate and easily interpretable estimate of risk of foodborne illness associated with raw dairy consumption in the state of California using publicly available data. The intended measurement of risk is number of cases per unit of food product sold per year in the state of California. The methods are intended to be replicable by any concerned citizen for any food product of interest.

Methods: Data on incidences of foodborne illness in the state of California were obtained from the Center for Disease Control's (CDC) Foodborne Outbreak Online Database (FOOD). Only searchable years were utilized (1998-2009). Dairy exposure was taken from Headrick et al. (1997) – a report on raw dairy consumption in California obtained from the 1994 California Behavioral Risk Factor Surveillance System Survey. The California Department of Food and Agriculture reported to Headrick et al. that sales of fluid raw dairy for the year 1994 equaled 1.5 million gallons or 0.2% of all fluid milk sales in the state. We assumed consistent annual sales for the 12 year period under study.

Results: There were 2,252 illnesses associated with pasteurized dairy products in the defined study period, and 186 illnesses associated with upasteurized dairy (49 when excluding cases associated with queso fresco). Assuming consistent annual fluid milk sales in the state of California of 1.5 million gallons of unpasteurized milk per year and 748.5 million gallons of pasteurized milk per year, the 12-year incidence of foodborne illness associated with pasteurized dairy was 0.25 cases per million gallons of fluid milk sold (90% CI: 0.24, 0.26), and for unpasteurized dairy it was 10.33 (90% CI: 9.09, 11.58) cases per million gallons of milk sold. When excluding cases associated with queso fresco, the 12-year incidence was 2.72 (90% CI: 2.08, 3.36) cases per million gallons of milk sold. Relative risks when including and excluding queso fresco associated cases are 41.21 (90% CI: 37.54, 44.64) and 10.86 (90% CI: 8.60, 12.96), respectively.

Significance: The choice to consume raw dairy in the state of California comes with a demonstrable excess risk of foodborne illness. Even when discounting all cases associated with queso fresco from the numerator, the relative risk of consuming raw dairy rather than pasteurized dairy in the state of California is approximately 10.

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P1-128 Identifying Turkish Pine Nuts Linked to an Outbreak of *Salmonella* Enteritidis: Using Product Trace-back in Conjunction with Laboratory Data

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Introduction: In October 2011, CDC notified FDA of an outbreak of a rare strain of *Salmonella*Enteritidis infections that was linked to consumption of bulk Turkish pine nuts (TPN), or products prepared with bulk TPN, purchased at Grocery Store Chain A. Forty-three individuals from 5 states (MD, NJ, NY, PA, and VA) were identified.

Purpose: To determine the supply chain of TPN to Grocery Store Chain A and product lots potentially associated with illnesses.

Methods: Case clusters identified by CDC were included in the traceback investigation based on the following criteria: geographic distribution, case exposure dates, and laboratory findings. FDA and state partners collected shipment records at the points of service, distribution centers, and importer. The records were reviewed and analyzed to determine product identifying information along the supply chain. Federal and state partners tested regulatory and retail samples of TPN collected along the supply chain.

Results: Grocery Store Chain A received bulk TPN solely from Distribution Center B. Distribution Center B received product from a single Importer C, which is supplied by two foreign suppliers (D and E) located in Turkey. Two intact samples of TPN collected along the supply chain, one open retail sample, and multiple open samples from consumer homes tested positive for the same strain of *Salmonella* Enteritidis found in clinical cases. Records collected provided the following information for traceback identification of TPN lots associated with illness: 1) purchase dates, 2) shipment frequency to points of service, and 3) availability of lots stored in distribution center and importer warehouses. The overall investigation of TPN led to the implication of 3 lots by traceback investigation and 1 lot by positive laboratory results.

Significance: The traceback investigation performed by FDA and state partners along with laboratory sampling led to the voluntary recall of 21,000 lbs. of TPNs by Importer C. FDA placed Foreign Suppliers D and E on import alert.

P1-129 Immunoreactivity of Hypoallergenic Peanuts Produced Using the Duodenal Endopeptidases Trypsin and α-Chymotrypsin

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Introduction: Peanuts (*Arachis hypogaea*) are a highly valued food source, rich in micronutrients, protein, and fiber. However, extensive use of peanuts in the food industry is limited because of their ability to cause allergic reactions in 1-2% of the US population. Recently, the Ahmedna group has reported on the ability of the duodenal endopeptidases trypsin and alpha-chymotrypsin to reduce and/or eliminate allergens contained in whole peanuts.

Purpose: To critically assess the enzymatic approach used by the Ahmedna group to reduce and/or eliminate the allergens contained in whole peanuts or peanut derivatives.

Methods: Peanuts (Spanish and Runner cultivars) were sanitized and then treated to enzymatic digestion using a panel of endopeptidases under optimized conditions. In some instances, enzymes were applied to homemade peanut butter or to extracted peanut proteins directly. Following treatment, nuts were rinsed, heated overnight at 70°C and then ground into a homogenous paste. Proteins were then isolated using a standard extraction protocol. Assessment of enzyme activity against the major peanut allergens, Ara h1, Ara h2 and Ara h3, was performed using a combination of SDS-PAGE electrophoresis, western blotting, and ELISA techniques. Assessment of immunogenicity was performed using pooled sera obtained from peanut-allergic patients.

Results: Protein gel analysis of the allergen profile of whole nuts following enzymatic treatment with trypsin and α-chymotrypsin demonstrated only a modest reduction of Ara h1, Ara h2 and Ara h3 bands. Furthermore, assessment of the immunoreactivity of these bands and their subsequent cleavage products using ELISA and western blot analysis revealed the overt presence of immunoreactive peptides.

Significance: Enzymatic digestion of allergens contained in whole nut matrix of peanuts using trypsin or α-chymotrypsin is incomplete at best, and results in the generation of peptides that fully retain immunogenic epitopes capable of eliciting allergic responses. These results indicate that additional approaches are required that are aimed at reducing the immunogenic potential of peanut allergens.

P1-130 Occurrence of Aflatoxigenic *Aspergillus* Species in Corn Harvested from Different Locations in Korea

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Introduction: Corn is one of cereals vulnerable to aflatoxin contamination. In South Korea, low levels of aflatoxin have been detected in corn and corn-based products, but information about the fungi involved in this contamination is limited.

Purpose: The aim of this study was to determine, identify and characterize the occurrence of aflatoxin-producing species in corn harvested from 25 locations in South Korea.

Methods: A total of 216 fungal isolates were obtained from 66 corn samples in Korea collected in 2010. After incubation in pure culture, aflatoxin production in those isolates was investigated via multiplex PCR assay, thin layer chromatography and high performance chromatography. Repetitive sequence based PCR (rep-PCR) DNA fingerprinting was also performed to determine genomic relationships.

Results: A range of 0-1.5×10^6 CFU/g fungal flora counted from corn samples. Mycological analyses showed that *Fusarium* and *Penicillium* species (22.2%) were the most predominant species, followed by *Mucor* species (12.0%), *Aspergillus*species (11.1%) and other fungal genera. Out of 24 *Aspergillus* isolates, 14 isolates of *Aspergillus* section flavi were screened through morphological and genetic identification. Among 14 isolates, three isolates showed complete amplified patterns in all target genes, and produced aflatoxin B1. Rep-PCR DNA fingerprinting showed that five isolates from corn were clustered with aflatoxigenic *A. flavus*KCCM60330 (98-98.8% similarity).

Significance: These results suggest that occurrence and abundance of aflatoxigenic *Aspergillus* in corn were low.

P1-131 Toxicity Profile of Commercially Produced Indigenous Rwandan Banana Beer 'Urgwagwa'

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Introduction: Mycotoxins together with endotoxins represent important classes of naturally occurring contaminants in food products, posing a significant health risks to consumers.

Purpose: The aim of this study was to investigate the occurrence of both *Fusarium*-mycotoxins and endotoxins in commercially-produced traditional Rwandan banana beer.

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Methods: Two brands of commercially-produced traditional banana beer were collected from local retail market in Kigali, Rwanda. Beer samples were analyzed for the presence of deoxynivalenol (DON), fumonisin B_1(FB1) and zearalenone (ZEN) using an enzyme-linked immunosorbent assay (ELISA) method. The quantification of bacterial endotoxin using Limulus Amoeboecyte Lysate (LAL) assay was also conducted.

Results: The contamination levels were 20 ppb and 6.7 ppb for DON; 34 ppb and 31.3 ppb for FB1; 0.66 ppb and 2.2 ppb for ZEN in Brand A and B of the beers, respectively. Results indicate that the levels of *Fusarium*-toxins and bacterial endotoxin reported in this study did not indicate adverse human health effects as result of drinking/consuming banana beer. However, exposure to low/sub-threshold doses or non-toxic levels of endotoxins magnifies the toxic effect of xenobiotic agents (eg., fungal toxins) on liver and other target organs.

Significance: Considering *Fusarium*-toxins and/or endotoxin contamination levels in other agricultural commodities intended for human consumption health risks might be high, and the condition is aggravated when beer is contaminated by mixtures of the mycotoxins as indicated in this study.

P1-132 A Seven-year Study (2005-2011) on the Occurrence of Patulin in Juices and Pulps of Different Fruits in Argentina

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Introduction: Patulin is a mycotoxin produced by a diversity of molds, particularly *Penicillium* and *Aspergillus*and mainly found in pomaceous fruits, such as apples and pears. Although patulin has been shown to present a genotoxic activity, its presence in fruit products has concerned much more to the quality point of view than health aspects. This is because high levels of patulin in juices indicate that moldy fruits were used in their production.

Purpose: This study aimed at quantifying patulin in fruit juices produced and/or commercialized in Argentina during a period of seven years.

Methods: Between 2005 and 2011, a total of 3,752 samples of juices (single strength, concentrated cloudy and clarified, and pulps) of apples, pear, peach, apricot, grape and pineapple were analyzed for patulin. The quantification of the mycotoxin was performed using a HPLC method with limit of detection and quantification of 3 and 10 ppb, respectively.

Results: Patulin was quantified in 373 out of 3,097 samples of apple juice and juice products analyzed in levels > 50 ppb. Pear juices and pulps were also positive for patulin (19 out of 350), while peach, apricot, grape and pineapple were negative for patulin (< 3 ppb). Among the positive samples, most presented levels of patulin between 50-60 ppb, however, few samples (n=94) presented levels as high as > 100 ppb.

Significance: The results indicate that intervention strategies must be improved in order to reduce the amount of patulin in apple juices. Strategies should include reduction of contamination of fruits in the field and growth of mycotoxin-producing molds during storage. Peach, apricot and grape do not represent sources of patulin in diet.

P1-133 Occurrence of Ochratoxin A in Fruit Juices and Wine in Argentina between 2005 and 2011

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Introduction: Ochratoxin A (OTA) one of the most abundant mycotoxins contaminating foods in the world. OTA is produced by some species of the genera *Aspergillus* and *Penicillium*, and it has been found in several foods. OTA presents nephrotoxic, immunotoxic, genotoxic and carcinogenic effects and its presence in foods has been regulated worldwide.

Purpose: The purpose of this study was to quantify OTA in fruit juices and wine originating from different provinces of Argentina between 2005 and 2011.

Methods: A total of 955 samples of grape juice (single strength, concentrated and sulfitated juices) and wines originating from five different Argentinean provinces were collected between 2005 and 2011. Cleanup step was carried out using an immunoaffinity column, followed by the determination of OTA in the samples through HPLC. The limits of detection and quantification were 0.15 and 0.3 ppb, respectively.

Results: OTA was not detected in any of the 620 samples of wines collected during the six years of study. On the other hand, this mycotoxin was detected in 5 samples (out of 29) of single strength grape juice, in 3 samples (out of 105) of concentrated clarified grape juice and in 7 (out of 201) of sulfitated juice. In the positive samples, the level of OTA varied from 0.22 to 3.6 ppb.

Significance: The results obtained in this study indicate good quality of the raw materials used in the processing of wine. The low levels of this mycotoxin in grape juices indicate that preventive measures need to be adopted to safeguard consumer's exposure to OTA as low and continuous exposure to this mycotoxin could be a risk to human health.

P1-134 Distribution of Fusarium Mycotoxins Deoxynivalenol and Zearalenone in Milling Fractions of Rice (*Oryza sativa*) Harvested from Korea

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Introduction: Korea, where rice is the cereal most consumed, has set maximum limits for *Fusarium*mycotoxins deoxynivalenol (DON) and zearalenone (ZEN). The maximum permitted level decreases from unprocessed rice, through intermediary products, to finished products. It is, therefore, important to understand the effects of milling process on the mycotoxin distribution.

Purpose: In this study, the fate of DON and ZEN during milling of Korean rice cultivars was investigated.

Methods: Rice (n = 80) grown under different field production conditions were collected and milled using a laboratory-scale test mill to produce four fractions: white rice, bran, brown rice and hulls. The four milling fractions were analyzed for DON and ZEN with a validated analytical method using high-performance liquid chromatography (HPLC) with UV absorbance and fluorescence detection.

Results: The contamination levels in white rice, bran, brown rice and hulls were 0, 0.5, 4.1 and 1.9 μg/kg for DON, and 0.6, 33.0, 156.6 and 923.1 μg/kg for ZEN, respectively. Among 320 milling fractions, the incidence of DON was ranged from 0 to 8.8%. In contrast, ZEN was detected in all milling fractions, but exponentially reduced through milling process.

Significance: Understanding the effect of milling that impact on the fractionation of DON and ZEN milling will help rice processors and/or risk managers to control rice-based products within legislative limits.

P1-135 An Experimental Study on Aflatoxin M1 Binding by Probiotic Bacteria in Yogurt

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Introduction: Mycotoxins are of great concern because of their acute and long-term toxicity. Aflatoxin M1 is an important mycotoxin frequently found in milk and dairy products. The exposure of some bacterial strains might affect and cause a reduction in the concentration aflatoxin M1.

Purpose: The aim of this study is to investigate and understand the behavior of two probiotic bacterial strains; *Lactobacillus acidophilus* LA5, *Bifidobacterium animalis/lactis*BB12 in corporation with yogurt starters to eliminate aflatoxin M1 in yogurt.

Methods: High Performance Liquid Chromatography equipped with fluorescence detector was used to determine Aflatoxin M1 in yogurt. The method was optimized and validated according to Commission Decision BS EN ISO 14501:2007 by using the conventional validation approach, according to the standard, selectivity, recovery, and precision of the method.

Standard samples of known aflatoxin M1 concentrations (0.1 to 10 µg/l) were injected and a linear calibration curve was obtained. In order to validate the method, the mean recoveries were calculated at three different levels of aflatoxin M1 additions (0.5, 1.0, and 1.5-fold the MRL).

Later reconstituted milk with added aflatoxin M1 at concentrations of 0.050 and 0.100 µg/l was fermented with two probiotic bacteria (*Lactobacillus acidophilus* LA5, *Bifidobacterium animalis*BB12) in corporation with yogurt starter cultures to reach pH value of 4.6. The prepared yogurt samples were stored at 4 °C for 4 weeks. Qualitative and quantitative tests concerned with aflatoxin M1 were performed by the application of HPLC using validated method.

Results: The results show that the calibration curve was linear for the concentrations of 0.1–10 µg/l when injected. The average recoveries were determined on three different days for various concentrations (0.025, 0.050, 0.075 µg/kg) in septimal orders (72.57 – 86.66%) with RSD in the range of 2.56 – 8.41 %. The limit of detection (LOD) and limit of quantitation (LOQ) values were 0.006 µg/kg and 0.015 µg/kg, respectively. The method was not affected by slight variations of some critical factors as pre-treatments and different storage conditions.

The results of the validation and the method used are in agreement with method described by Commission Regulation 401:2006: EC which might be regarded rapid, reproducible and simple to apply.

AFM1 levels in yogurt samples showed a significant decrease ($P < 0.01$) as compared to those initially added to milk. The growth of lactic acid bacteria was not affected by the presence of aflatoxin M1 except *Streptococcus thermophilus* that showed a significant ($P < 0.01$) decrease in the yogurt containing the toxin at high concentration. During the course of fermentation, AFM1 was significantly decreased ($P < 0.01$) in yogurts at both contamination levels; however, storage at refrigerated conditions did not affected the levels of aflatoxin significantly ($P > 0.01$). Final loses of 33 to 39% were observed at the end of both fermentation and refrigeration practice.

Significance: It therefore might be suggested that the strains of probiotic bacteria which were studied in this research work and are currently used in the food industries, due to their cost-effective and detoxification potentials employed at great demand for decreasing of aflatoxin M1 level in milk products.

P1-136 Isolation and Characterization of Emetic Toxin Producer *Bacillus cereus* from Milk Tea

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Introduction: In Taiwan, *Bacillus cereus* is one of the major foodborne agents of food poisoning outbreaks (125 outbreaks, during 2000-2010), and can cause diarrhea and emetic type of foodborne illness. Soft drinks made by convenient stores are popular, but they cause outbreaks with high dose and low dose of detected *Bacillus cereus* from time to time. *Bacillus cereus* positive samples can be determined their diarrhea toxins by commercial reverse passive latex agglutination (RPLA)test kit. However, none of these involved samples and strains have been determined their emetic toxin expression (cereulide) in Taiwan.

Purpose: The object of this study is to investigate the emetic toxin producing strains in soft drinks, and evaluate the toxin expression ability of these strains in culture media and food sample.

Methods: By using LC-MS method to detect emetic toxin, BCRC 17039 (ATCC 14579, cereulide producing strain), BFDA 3511, BFDA 5113, and BFDA 5511 strain can produce cereulide 347, 164, 176 and 217 ppm (TSA, 25¢J, 10 days), respectively.

Results: Results showed the detected rate of *Bacillus cereus* in soft drink is 19% (29/150), and microorganisms range between 3~460 MPN/g. Among the selected *Bacillus cereus* (N=51), there are 3 strains (BFDA 3511, BFDA 5113, BFDA 5511) which isolated from 3 *Bacillus cereus* positive samples (milk-tea with herb jelly, pearl jelly and coconut jelly, separately) containing with emetic toxin producing gene. The data also revealed all 3 milk-tea isolates produced more cereulide than BCRC 17039 and BCRC 10603 (ATCC 14579, type strain) in the cooked rice after 24 hrs incubation. Furthermore, all emetic producing strains in this study, the high dose inoculation (10^6 CFU/g, 24 hrs) in cooked rice incubate in 25¢J produced more cereulide than in 35¢J.

Significance: According to the survey, the detected microorganisms in positive samples are in low levels yet, there is emetic toxin producer *Bacillus cereus*. Moreover, these strains can produce emetic toxin in room temperature. Therefore, the temperature control of milk-tea would be one of the key factors to decrease the risk of *Bacillus cereus* emetic toxin outbreak.

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P1-137 Rapid Detection and Discrimination of *Bacillus* Species Using Immunomagnetic Separation Combined with Surface-enhanced Raman Spectroscopy

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Developing Scientist Competitor

Introduction: *Bacillus* species are Gram-positive, spore-forming bacteria which include *B. anthracis*, a Class A bioterrorism agent according to the Centers for Disease Control and Prevention and the Department of Homeland Security. Because of the potential high risk of anthrax spores, it is important to be able to quickly and accurately detect anthrax spores in environmental and food samples.

Purpose: This research aimed to discriminate between three *Bacillus* species, *B. anthracis*, *B. thuringiensis*, and *B. mycoides* using SERS and establish an IMS-SERS procedure that can detect *B. anthracis* spores in milk within 20 minutes.

Methods: In this study, Surface-Enhanced Raman Spectroscopy (SERS) was first utilized in order to discriminate between *Bacillus* species. In order to detect *B. anthracis* in food systems, immunomagetic separation (IMS) was used to capture out of solution. This is then treated with dodecylamine, which digests the sporal coat. The resulting solution is analyzed for the spore biomarker dipicolinic acid (DPA).

Results: The results show that *Bacillus* species and their cell states (live cell, dead cell, spore) could be differentiated when SERS spectra were analyzed using principal component analysis and hierarchical cluster analysis. The limit of detection was $2x10^7$ spores/ml due to the difficulty to optically locate the spores under a Raman microscope. In order to achieve a lower limit of detection, the spores were captured by IMS. This method was shown to have a ~50% recovery of *B. anthracis* spores from water and milk within 15 min. The spores captured by IMS were treated with dodecylamine, which allowed a lower limit of detection at $2x10^3$spores/ml within 20 min.

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Significance: Based on published toxicological data, detection at this limit is sufficient. This method could be extended to detect other spore-forming bacteria and in a variety of matrices.

P1-138 A Comprehensive Curriculum to Prepare Consumers to Keep Food Safe before, during and after a Disaster

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Introduction: When disaster strikes, there is an increased risk of contracting foodborne illness. Power outages make it difficult to keep perishable foods cold and to cook foods to proper temperatures. Flood waters or environmental substances may contaminate food and make it harmful to eat. Consumers need to be prepared for such emergencies and the possibility of foodborne bioterrorism in order to have food available and safe.

Purpose: To develop and disseminate a comprehensive educational curriculum based on results of consumer focus groups and national survey data.

Methods: Using information from USDA, FDA, Red Cross and FEMA, we developed a booklet with basic information on how to prepare for and keep food and water safe before, during and after disasters. An 8-lesson curriculum designed for use by extension or other community personnel was developed and reviewed by professionals. Each lesson supports an educational approach that is in-depth, interactive and flexible to the specific educational setting. DVDs with the full curriculum were prepared.

Results: The curriculum was demonstrated at a state family and consumer science (FSC) extension workshop. DVDs with the curriculum were distributed at the conference or mailed to FCS agent or county directors and distributed to all North Carolina extension offices. Additionally, the curriculum is available nationwide to EDEN network members and on the TSU website. Based on anecdotal feedback, the DVD was well received by educational and other professionals.

Significance: Consumers need to be prepared for food-related emergencies. Educators can use this curriculum to deliver effective messages on how to prepare and respond to disasters to ensure food safety. Because the DVD provides everything needed to successfully teach the curriculum, it should become a highly-sought after addition to educators' and community personnel's repertoire of educational materials. Attendees will receive a copy of the DVD at the presentation.

P1-139 The Impact of a Food Hygiene Training Program on Foodservice Staff in Saudi Arabian Hospitals

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Developing Scientist Competitor

Introduction: Foodservice departments in hospitals are required to provide patients with meals prepared under strict hygiene conditions because patients have a weakened immune system. Foodservices staff must have adequate hygiene training and sufficient knowledge about good practices. Lack of training may cause cases of food poisoning which could have serious consequences for patients who are already ill. In Saudi Arabia, although training programs for foodservice staff is still limited, the Ministry of Health intends to implement HACCP systems in hospitals. It is likely that the current level of knowledge will need to be augmented before HACCP can be successfully implemented in all hospitals.

Purpose: The main aim was to assess the knowledge, practices and attitude of hospital food handlers in Riyadh, to develop a bespoke training programme and to evaluate the effectiveness of this training.

Methods: The study was a longitudinal study which assessed the staff knowledge at the pre-training stage by using a multiple choice questionnaire. This data was analyzed to identify the specific deficits in food safety knowledge and practices of staff and the training program was developed according to the weak points. After delivering the training program participants were reassessed. The sample comprised 129 food handlers in 3 large hospitals in Riyadh. The questionnaire contained 32 questions and was administered in Arabic, English, Bengali and Urdu languages. All analyses were performed by using SPSS software (version 18)

Results: Approximately 60% of participants were male with an average age of 25 years. Over 50% were educated only to the level of elementary school. The mean knowledge score before attending the session was poor (36%) but increased significantly after attending the training (75%, $P < 0.001$). Self-reported practices and attitude were also assessed and these too improved significantly after the training ($P < 0.001$).

Significance: This data suggests that food service handlers in Saudi Arabian hospitals respond well to bespoke hygiene training. Such training can be used to support the Ministry of Health in Saudi Arabia when implementing HACCP in hospitals.

P1-140 Integration of Pulsed Field Gel Electrophoresis Technology into an Undergraduate Food Science Curriculum

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Introduction: The food industry and regulatory agencies are increasingly using molecular-based detection and characterization methods to identify pathogens that cause foodborne disease outbreaks. Students preparing for careers in food safety need access to curricula that develops skills enabling them to execute and understand these methods, which significantly enhance food safety.

Purpose: The goal of this study was to introduce Pulsed Field Gel Electrophoresis (PFGE) and PulseNet into the undergraduate Food Science curricula at Purdue University. PFGE is considered the gold standard molecular subtyping method and is used by regulatory agencies in the PulseNet network to detect foodborne disease outbreaks.

Methods: Outcome-based learning objectives were defined prior to instruction. New curricula materials were developed and implemented concurrently in food microbiology lecture and laboratory courses for a two week period. In addition to classroom lecture instruction, the curriculum included hands-on execution of the Centers for Disease Control PFGE protocol for *Listeria monocytogenes*fingerprinting. Pre- and post-instruction assessments were used to quantify learning gains in both courses.

Results: Student assessment scores significantly increased from mean pre-instruction score of 9.7% (1.7/17) to mean post-instruction score of 75.7% (12.6/17) (n=56, $P < .0001$). The vast majority of students (98.2%) demonstrated improvement in post-instruction assessment scores in the lecture course; the mean score improvement was 10.90 ± 0.52 points. Laboratory post-instruction assessment scores improved from an average score of 58.7% to 97.2% (n=45, $P < .0001$); 97.8% of the students improved their scores; mean scores improved by 38.5%.

Significance: This study demonstrated successful integration of PFGE and PulseNet technology into undergraduate food science curricula. Lecture and laboratory exercises resulted in positive learning outcomes and enhanced students' preparedness for successful food safety careers.

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P1-141 Development of an Effective Mechanism to Improve Food Safety and Quality Standards in Small and Medium Sized Enterprises (SMEs) in the Manufacturing and Processing Sector

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Introduction: The Food and Drink Manufacturing and Processing (FDMP) industry is a fundamental element of the food supply chain in Wales (UK), the majority (98%) of FDMP businesses are SMEs; compliance to food safety regulations and obtaining 3rd party accreditations are essential for business sustainability and growth. However, reports indicate a lack of necessary scientific/technical skills to deliver the business needs.

Purpose: This study aimed to develop a model to improve food safety and technical compliance in SMEs and increase food science/technology knowledge in the FDMP sector.

Methods: At the concept development stage, a needs analysis determined technical support/requirements of FDMP SMEs in Wales. Model design was based on assessment of food-sector diagnostic technical evaluations (n=90) and consultation with SMEs (n=~200). The model was piloted in two SMEs ('established' and 'start-up' stages of business) over 12 months to determine operational feasibility and potential effectiveness.

Results: A review of diagnostic technical evaluations (n=29,350) (benchmarked against BRC5), showed > 40% non-conformities were HACCP related, e.g., inaccurate CCP definition (23%), poor allergen-control management (58%) and lack of training (31%). Barriers to technical innovation were largely resource related. SME consultations established a critical need and significant demand for an outlet to supply food science/technology expertise with long-term support. The KITE (Knowledge, Innovation, Transfer, Exchange) Model was designed to meet these needs and was based on a collaborative partnership: an industrial partner (SME), a knowledge-based partner and an affiliate (graduate/individual with industrial-experience) placement. Piloting facilitated evaluation of KITE operational processes and resulted in achievement of global/retail food safety/quality standards; new product development; upskilling technologists and embedding knowledge in an existing and new workforce; increased employment; sales and overall improved business sustainability. Difficulties encountered during pilot implementation will be presented in the framework of technical management and historical food safety culture.

Significance: Development of the KITE model has created an effective mechanism tailored to the needs of FDMP SMEs by embedding food science/technology knowledge, increasing innovation, improving technical compliance and increasing potential business sustainability. Development and implementation of KITE will be discussed in the context of international application in the food industry.

P1-142 Experiences of Food Allergy Sufferers with College and University Dining Services

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Introduction: Food allergy is a response of the immune system to proteins in a food item and affects 2 to 4% of adults and approximately 2.3% of teenagers in the United States. The eight major food allergens are milk, eggs, peanuts, shellfish, tree nuts, soy, fish and wheat and are responsible for 90% of the allergic reactions. An allergic reaction includes rashes, hives, tingling sensations, trouble breathing, anaphylactic shock and even death. It was found that 16 out of 63 food allergy fatalities happened to college-aged students with 50% of the fatalities happening on college campuses.

Purpose: The purpose of this study was to determine the experiences of food allergic students with college and university dining services.

Methods: A 47-item survey was posted on the Food Allergy and Anaphylaxis Network (FAAN) and Food Allergy and Anaphylaxis Network Teen (FAAN Teen) Facebook pages in addition to distribution via email to students at two major universities in Midwestern United States. The questionnaire explored general perceptions about food allergy accommodations at their college/university, respondents' comfort levels with dining at college/university foodservice establishments, in addition to demographic information. Data was analyzed using SPSS 20.0, and descriptive statistics were calculated.

Results: Results indicate that college and university students suffering from food allergies are satisfied with the accommodations provided by college and university dining services. However, there is a need for educating foodservice workers about proper handling of allergen-containing foods, distinguishing between food allergies and food intolerances, and providing easy to read food allergen information at dining establishments.

Significance: Outcomes from this study will help identify the needs of food allergic students and improve college and university dining foodservice operations to avoid incidents of allergic reactions or anaphylaxis.

P1-143 Development of the Food Safety Wiki Aims to Provide Comprehensive Information and Resources about Food Safety Education

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Introduction: We developed an integrated approach to promote food safety education and training opportunities via the world wide web. There are currently very limited efforts to interest students at the K-12 and undergraduate level, and specifically to recruit graduate students into food safety related programs. We thus developed a Food Safey Wiki that includes comprehensive food safety education information for K-12 teachers, food safety undergraduate and graduate programs, and training opportunities for current food safety professionals.

Purpose: Our purpose was to develop a Food Safety Wiki with content that focuses on food safety education and opportunities from K-12 through graduate school and on to training for current food safety professionals. The Food Safety Wiki is an easily accessible resource for anyone interested in or needing food safety information.

Methods: The Food Safety Wiki was developed using the Confluence software by Atlassian. This effort was spearheaded by Cornell University, while collaborators at Colorado State University, Purdue University, North Carolina State University, Alabama A&M University, North Carolina A & T State University, Texas Tech University and Texas Wesleyan University assisted in contributing materials for the website.

Results: The Food Safety Wiki (available at: https://confluence.cornell.edu/display/ FOODSAFETY/Welcome+to+Food+Safety+Wiki!) is a comprehensive resource for those involved in varying levels of food safety education. The Food Safety Wiki contains up-to-date information on K-12 teacher resources and workshops, Universities with Food Safety programs (e.g., links to application materials and specific faculty members with food safety expertise), food safety conferences, internship opportunities, job opportunities, listservs and blogs, food safety publications, databases (i.e., Pathogen Tracker), and professional associations. The Food Safety Wiki also contains information on specific foodborne pathogens and lists laboratories throughout the world that work on each pathogen.

Significance: The Food Safety Wiki is an easily accessible resource that will link different food safety efforts and institutions and will facilitate dissemination of information on food safety and foster early interest in food safety careers among K-12 through graduate students.

Poster

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P1-144 Organizational Climate and Food Safety Training Change Employee Behaviors and Pathogen Loads in Non-inspected Beef Packing Plants in Mexico

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Introduction: As foodborne illness is the number one cause of death in children under five in Mexico, food safety knowledge and practices in Mexico need improvement. In non-inspected (non-Tipo Inspección Federal, non-TIF) meat packing plants in Mexico, food safety is considered a low priority. Training non-TIF management and employees in basic food safety and carcass dressing procedures could significantly reduce the prevalence of *Salmonella* in the Mexican food supply.

Purpose: Examine *Salmonella* prevalence on carcasses in non-TIF beef packing plants in Mexico as affected by manager and employee training.

Methods: Organizational climate and food safety knowledge surveys were administered to the management and employees of two non-TIF plants. GMP audits were conducted, and microbial testing of carcasses served as baseline sampling for *Salmonella* contamination. Food safety training gaps were assessed using audits, surveys, and microbial data. Two trainings were provided with longitudinal data recorded following each session. The first workshop was administered to managers at both plants, while the second was provided for line workers at one plant. An average of 10 post-evisceration samples were taken from both plants at each of four seasons. The workshop for managers was conducted between the spring and summer collections. Line worker training in Plant 2 took place between summer and fall.

Results: Carcasses from Plant 2 had higher *Salmonella* prevalence rates than Plant 1. In the winter, Plant 2 prevalence was 68.5% higher than Plant 1; spring, 58.4%; summer, 68.7% and fall 38.0%. ANOVA indicates that the 38% difference in prevalence between Plants 1 and 2 in the fall after line worker training was significantly lower than the difference in prevalence at the other three data collection periods.

Significance: Management training had no impact on *Salmonella* prevalence, while line worker training reduced prevalence significantly. This knowledge can be used in future trainings to improve the safety of the Mexican food supply and reduce the incidence of foodborne illness.

P1-145 Determination of Microbiological and Behavioral Risks Associated with Listeriosis in Older Adults' (> 60 Years) Domestic Kitchens

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Introduction: Listeriosis has the highest rate of reported hospitalizations (91%-95%) and related mortality (20%-40%) of foodborne diseases in Europe and USA. European incidence has doubled since 2001, almost exclusively among adults aged >60 years. The domestic kitchen is a potential source of sporadic foodborne illness. Reports indicate older adults consume more ready-to-eat (RTE) foods commonly associated with *Listeria monocytogenes*prevalence than other consumer groups, therefore implementation of safe domestic food-handling/storage is required to reduce the risk of listeriosis.

Purpose: This study aims to evaluate microbiological risks factors in older adults' domestic kitchens that may contribute to listeriosis.

Methods: Food contact surfaces (n=984) in older adults' (> 60 years) domestic kitchens (n=99) were assessed for presence of *Listeria* spp. and *L. monocytogenes*. Refrigerator temperatures were recorded and food safety/storage behavior risk factors associated with listeriosis were observed and recorded in a structured survey.

Results: *Listeria* spp. was detected in 7% of domestic kitchens, predominately from wet environments including the sink and dishcloth. *L. monocytogenes* was isolated in 2% of kitchens, on a refrigerator door handle and a hot water tap handle. Ninety-three-percent of domestic refrigerators operated at temperatures exceeding 5°C, with some temperatures ranging up to 17.4 °C (mean 6.2°C). The majority (70%) of refrigerators contained foods associated with *L. monocytogenes*, of which, 54% had been stored longer than the recommended 2 days. Food products beyond 'use-by' dates, reportedly intended for consumption, were stored in 43% of refrigerators. Foods associated with listeriosis such as soft cheese, butter and cooked meat were observed being stored at room temperature, by 15% older adults for reportedly up to 4 weeks.

Significance: Behavioral and microbiological findings suggest older adults (aged 60 > years) frequently implement unsafe food storage practices that increase the risk of listeriosis in the domestic kitchen. Findings may be used to inform the development of targeted consumer food safety interventions to increase implementation of safe food-handling/storage behaviors to reduce listeriosis risk.

P1-146 A Comparison of Food Safety Climate at Municipal and Private Beef Slaughter Plants in Mexico

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Introduction: Worker behavior in beef packing plants has a direct impact on product quality and safety. A key factor in explaining worker behavior related to food safety procedures is organizational climate. The food safety climate within an organization must be assessed in order to interpret its relationship with food safety training effectiveness.

Purpose: Using a food safety climate survey instrument, this research sought to determine differences in food safety climate (worker attitude regarding food safety) between a municipal beef slaughter plant and a private beef slaughter plant in Mexico. Additionally, researchers sought to determine in which constructs any identified differences occurred.

Methods: The data for this study was collected at two beef processing plants in Mexico. One plant was municipally owned and managed by the city government, while the other was a privately owned and operated plant. A 39-question survey instrument was administered to the employees at each plant, regarding their perceptions of food safety climate within their slaughter plant. Instrumentation was provided in Spanish and in necessary situations, the instrument was read to the participant and responses were recorded by the researcher.

Results: An ANOVA was conducted to measure the results of the climate survey between the municipal and private plant, determining significant differences in several construct areas. The private plant scores were significantly higher (more positive in relation to food safety) in areas including Food Safety Training, F (2, 203) = 9.12, P = .01; Management Commitment, F (2, 121) = 22.02, P - > .01; and Worker Behavior, F (2, 127) = 19.27, P= > .01) indicating a more willing environment to effect changes in behavior

Significance: Food safety climate within an organization is a key factor in explaining worker behavior related to producing a safe product. Municipal plants in Mexico work within a model that fails to promote a healthy climate and, in turn, could have negative impacts on the effectiveness of training conducted for the employees. Understanding this climatic variance is an essential first step at developing training that will have the desired effects. Developing a targeted training plan with the organizational climate in mind is pertinent to providing a safe food product.

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P1-147 Assessment of Food Safety Practices of Older Adults (> 60 years) in a Model Kitchen

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Introduction: The domestic kitchen is an important point of origin for foodborne disease; safe food-handling/storage behaviors are needed to reduce sporadic incidence. European data suggest foodborne disease associated with older adults has increased (by up to 80%) over the last decade. Although older adults are more susceptible to foodborne disease, data suggest knowledge and attitudes towards food-safety practices are inadequate, which may influence implementation of unsafe food-handling/storage practices.

Purpose: Determine food-safety practices of older adults and link observed behaviors with potential routes of actual microbiological contamination.

Methods: Older adults (> 60 years, n=100) prepared a set meal in a model domestic kitchen, handling foods commonly associated with pathogen contamination and using high-risk practices. Food-safety practices were observed using ceiling-mounted digital cameras and recorded using a predetermined checklist. Prior to food preparation, food-contact surfaces were cleaned according to a validated protocol; post food preparation, surfaces were immediately sampled to determine aerobic colony count (ACC), *Enterobacteriaceae* and *Staphylococcus aureus*contamination.

Results: Cumulatively, observational findings indicate that older adults frequently implement unsafe food-handling practices that can be linked to microbial contamination of kitchen surfaces. Eighty-four percent of older adults failed to attempt/adequately implement hand-washing/drying immediately after handling raw chicken. Kitchen surfaces most frequently touched with potentially contaminated hands were tap handles (79%) and refrigerator-door handles (65%); these surfaces were found to be contaminated with < 2.08×10^6 CFU ACC, < 4.75×10^5 CFU *Enterobacteriaceae* and < 2.59×10^5 CFU *S. aureus*, suggesting potentially contaminated hands were the source of contamination. Chopping boards/knives were inadequately washed/dried on 82-85% of occasions; this resulted in microbial counts of < $1.73 \times 10^{6\ CFU}$ ACC, < 1.82×10^4 CFU *Enterobacteriaceae* and < 1.75×10^3 CFU *S.aureus*. Indicators of safe food-storage efficacy (such as failing to cover ready-to-eat food for storage) suggested widespread malpractices; findings will be discussed in the context of microbial risk.

Significance: Findings from this study indicate that a considerable proportion of older adults implement unsafe food-handling practices which can potentially result in cross-contamination of pathogens and increase foodborne disease risk. This link suggests a need for targeted risk communication and has implications for future consumer food-safety education initiatives.

P1-148 Effect of Multi-level Spanish Food Safety Training on Knowledge, Behavior and System Change

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Introduction: There is a critical need for development and evaluation of food safety educational programs targeting the Hispanic food worker. Hispanic employees comprise 22% of the food preparation and service industry. Despite the increasingly important role of Hispanic workers in the food industry, food safety training programs do not appear to be sufficiently effective at improving food safety practices and diffusing food safety knowledge within organizations.

Purpose: This project was undertaken to implement culturally sensitive and language appropriate food safety training interventions for Hispanic food workers and to determine their effectiveness related to knowledge gain, behavior change, and the exchange of food safety information.

Methods: Restaurants meeting selection criteria were invited to participate in multi-level food safety training consisting of on-site education, manager certification and community engagement. Pre and post-training interviews were conducted including questions on demographics, food safety knowledge, behavior and communication. Training topics included personal hygiene, cross contamination, proper heating, cooling and holding of food, approved source and self-auditing.

Results: One hundred sixty Spanish speaking food workers were trained at 17 restaurants. Eighty-two percent of the participants made positive food safety behavior gains from pre to post programming. Participants indicated appreciation for group learning, interactive activities and educational material. Food manager class assessment indicated 89% of the students obtained passing scores with an average score of 87% as compared to 40% in a traditionally structured class. Analysis of employee social networks indicated substantial change in socially based food safety information structures – especially the elevated role of certified managers.

Significance: Food safety training and program evaluation appears to demonstrate that the implementation of culturally sensitive and language appropriate interventions can be effective at producing changes in knowledge, behavior, and social structures related to the exchange of food safety information at the restaurant level.

P1-149 Retention of Food Safety Knowledge after Faith-based Organization Volunteer Training

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Introduction: Faith-based organizations (FBO) are common places where foodborne illness outbreaks occur. One potential reason may be the fact that large quantities of food are often prepared by untrained volunteers and members of FBOs.

Purpose: The purpose of this study was to evaluate long-term effectiveness of food safety training developed for FBO volunteers and to compare effectiveness of training with or without interactive games.

Methods: FBOs in hurricane prone states and KS were recruited for food safety training. Volunteers and untrained members of congregations were invited to participate in the training. A 20-minute video with or without interactive games was used for training. An identical test was developed to measure food safety knowledge and attitudes toward food safety and training and pilot tested before data collection. Descriptive statistics summarized the data and repeated measures of MANOVA were calculated to evaluate the differences between the pre-test (Pre) and two post-tests: Immediately after the intervention (Post1) and three months follow-up (Post2).

Results: A total of 263 individuals participated in one of training sessions and completed Pre and Post1. Of those, 173 participants completed 3-month follow up test (Post2). The overall attitudes toward food safety and training were not different across three different tests ($P > 0.05$). However, knowledge scores (30 points total) significantly increased after the intervention (Pre = 19.8 ± 4.1, Post1 = 26.0 ± 2.8, & Post2 = 24.4 ± 2.8, $P < 0.001$). Post2 knowledge score was significantly lower than Post1 ($P < 0.001$) indicating reduction of knowledge over time, but the mean knowledge score after 3 months was 4.6 points higher than the pre-test ($P < 0.001$). Despite increased interactions and repeated information transfers during interactive games, there were no significant differences in mean knowledge scores of three tests between groups with or without games ($P > 0.05$).

Significance: After one hour of food safety training utilizing media and interactive games, volunteers and members of FBOs gained food safety knowledge. Providing short but targeted food safety training may benefit not-for-profit groups such as FBOs to ensure food safety.

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P1-150 Modeling Growth/No Growth Boundaries of *Escherichia coli* O157:H7 on Polyethylene Cutting Boards

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Introduction: Pathogens on cutting boards can be transferred to meat surface, and *E. coli* O157:H7 is considered as one of the major pathogenic bacteria in meat processing.

Purpose: This study described a kinetic behavior of *E. coli*O 157:H7 and developed probabilistic model to calculate growth probabilities of the pathogen on cutting boards.

Methods: *E. coli* O157:H7 (4 log CFU/cm^2) was inoculated on polyethylene coupons (3 × 5 cm). The inoculated coupons were stored at 13, 15, 20, 25, 28, 30, 33 and 35 °C for 12 h, and *E. coli* O157:H7 cell counts were enumerated on McConkeyII with sorbitol agar every 2 h. Kinetic parameters ($_{max}$: growth rate (log CFU/cm^2/h), LPD: lag phase duration (h), and N_{max}: upper asymptote (log CFU/cm^2) were calculated with the modified Gompertz equation. The 0.5-log CFU/$cm^2$was used as a threshold to determine growth or no growth for 56 combinations (8 temperatures, 7 sampling times). The growth response data were analyzed with the logistic regression analysis to produce growth/no growth interfaces at 0.1, 0.5, and 0.9 of probabilities. The model performance was then validated with observed data.

Results: Obvious growth of *E. coli* O157:H7 on coupons were not observed during storage at 12, 15, 20 and even at 25 °C for 12 h, but cell counts of the pathogen increased (P < 0.05) at 28, 30, 33 and 35 °C with 4.31-7.20 h of LPD, 0.42-2.06 log CFU/cm^2/h of $_{max}$, and 5.7-7.1 log CFU/cm^2 of N_{max}, depending on temperatures. Growth/no growth boundaries of *E. coli* O157:H7 were produced at 0.1, 0.5 and 0.9 of probabilities with acceptable performance (concordance: 99.3 %, discordance: 0.6 %). In addition, validation for the developed model was acceptable.

Significance: These results should be useful in determining working temperature and time not to allow *E. coli* O157:H7 growth on cutting boards.

P1-151 Validation of a *Salmonella* Survival and Growth Model for Extrapolation to a Different Previous History: Frozen Storage

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Introduction: Frozen storage of chicken can reduce the risk of salmonellosis by killing or injuring *Salmonella*. Injured *Salmonella* exhibit longer lag phases but similar growth rates as uninjured *Salmonella*.

Purpose: The objective of this study was to use a predictive microbiology approach to assess the impact of freezing on the survival and growth of *Salmonella*on chicken.

Methods: This was accomplished by evaluating a USDA, ARS, Pathogen Modeling Program (PMP) Model for survival and growth of *Salmonella* on chicken skin for its ability to predict survival and growth of *Salmonella* on chicken skin that was frozen for 6 days at -20 °C and then stored at 5 to 50°C for 8 h. Experimental methods used to collect data for model development were the same as those used to collect data for *Salmonella*survival and growth following frozen storage; this was done to provide a valid comparison of observed and predicted values. Residuals from individual survival and growth curves were evaluated using the Acceptable Prediction Zone (APZ) Method.

Results: The proportions of residuals in an acceptable prediction zone (pAPZ) from -1 log (fail-safe) to 0.5 log (fail-dangerous) were acceptable (pAPZ > 0.682) for all survival and growth curves; the overall pAPZ for the test data was 0.846 (154/182). However, there was evidence that freezing injured *Salmonella* as the mean residual was -0.3 log under growth conditions, which was different (P< 0.05) from zero or the mean residual predicted by the PMP model.

Significance: Findings of this study indicated that the PMP model developed with uninjured *Salmonella* provided valid predictions of survival and growth of *Salmonella* injured by a previous history of frozen storage. Validation of predictive models for extrapolation to independent variables not included during model development (e.g., previous frozen storage) can save time and money by identifying conditions for which new models are not needed.

P1-152 Estimation of Consumption Patterns of Potentially Hazardous Foods Including Ready-to-Eat Foods in Korea

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Developing Scientist Competitor

Introduction: The purpose of an exposure assessment is to estimate the frequency and amount of each serving of a food that has the potential for microbial contamination, as well as the microbial contamination level in the food.

Purpose: To estimate the consumption patterns and food safety perceptions of 50 potentially hazardous foods (PHFs) in Korea.

Methods: A quantitative survey was performed to assess the consumption patterns of PHFs including ready-to-eat (RTE) foods by 1,000 adults over 18 years of age, who were randomly selected from six major provinces in Korea. The survey was performed twice in May and August Data were obtained by by trained interviewers in face-to-face interviews. A picture of each PHF showing the amount per serving was given to the respondents. Questions included perception of PHF safety, intake amount and frequency, purchase and consumption patterns, habit for dining out, etc. Statistical analyses were carried out using SPSS (P < 0.05).

Results: The highest-risk food was seafood, including mussels (87.4%), shellfish (86.8%), raw oyster (84.6%), sashimi (82.4%), sushi (82.6%), tuna sashimi (76.6%) and raw sliced beef (84.7%). Consumption of seafood was most frequent by the professional male group. Frequency of eating sashimi and sushi was high in the middle-age economically-stable group. The respondents living in small regions consume more PHF portions at once. The most frequently consumed RTE was fried chicken, followed by ham/sausage/bacon, fried rice, hamburger, sandwich, cream cake, and hotdog, which was consumed more frequently by people under 40 years of age and living in the city. People ate more often in Korean food restaurants on weekdays, while restaurants that served grilled beef and pork were preferred on weekends.

Significance: Food safety education regarding PHF risks is needed by those consumers who eat large amounts of PHF more frequently. The results of this study can be used for the exposure assessment element of PHF risk assessments.

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P1-153 Development of a QMRA Model for *Listeria monocytogenes* on Frankfurters to Determine Risk-based Critical Control Points at the Processing Level

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Introduction: Quantitative Microbial Risk Assessment (QMRA) is being increasingly used to provide sound food safety advice to risk managers. However, its integration into the primary risk management system for food safety, HACCP, has been limited. The ability to link the stringency of HACCP programs to food safety outcomes is critical to ultimately developing risk-based food safety systems.

Purpose: The overall goal is to develop a means to allow food producers to more effectively link HACCP plans to food safety risk management metrics. The specific objectives include developing a facility-specific product-pathogen risk assessment model for frankfurters and determining if sensitivity analysis can be used to identify risk-based Critical Control Points.

Methods: A six-module risk assessment model for *Listeria monocytogenes* in frankfurters was developed in Excel using @Risk. The exposure assessment for frankfurters processing was divided into six modules: Ingredients, Raw Product Processing, Cooked Product Processing, Distribution and Marketing, and Consumer. The output of the exposure model was then linked to the Dose Response Module based on the FAO/WHO *L. monocytogenes*model. Risk distributions were then simulated (10,000-to-1,000,000 iterations), and subsequently analyzed via sensitivity and "what-if" scenario analyses.

Results: The contamination level of *Listeria monocytogenes*at the final storage level at the processing facility was found to be similar to that obtained from the reference data. The sensitivity analysis suggested that besides thermal processing, two additional CCPs included the use of gloves to remove non-compliant products prior to packaging and the time of final storage as the critical factors in the cooked product processing module.

Significance: This study provides a quantitative risk-based approach to determine critical control points which could be used for the implementation of hazard mitigation strategies and other food safety risk analysis decision making. This work provides a pathway to link safe food production with public health.

P1-154 Nitrite Supplementation of Drinking Water Does Not Impair the Resistance of Mice to Intragastric Inoculation with *Listeria monocytogenes*

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Introduction: There has been public controversy regarding the use of nitrite in cured meat products. Many vegetables, including leafy greens, are high in nitrate. Once ingested the nitrate is reduced to nitrite by bacteria residing in the oral cavity or gastrointestinal tract. Recent evidence suggest that nitrite is an important signaling molecule in the cardiovascular tract. One reactive nitrogen intermediate (nitric oxide) is also an antimicrobial effector molecule and can have a selective effect on regulatory T lymphocytes.

Purpose: In this project, we examined the effect of nitrite supplementation on host defense using a mouse model of gastrointestinal listeriosis. Our hypothesis was that dietary supplementation of drinking water with sodium nitrite will not affect resistance of mice to gastrointestinal infection with *Listeria monocytogenes*.

Methods: Mice were given graded amounts of nitrite (25 to 50 mg/l) in their sole source of drinking water. Control mice received water without nitrite. Although we observed a dose-dependent decrease in water intake by mice given drinking water supplemented with $NaNO_2$, water consumption remained within the normal range for mice. We then assessed the effects of $NaNO_2$ supplemented drinking water on the severity of infection in C57BL/6 mice inoculated intragastrically with 10^6 CFU *L. monocytogenes*. Mice received the indicated amount of $NaNO_2$ in their drinking water for 4 days before inoculation with *L. monocytogenes*, and throughout a 3-day period of infection. The mice were then euthanized, their tissues removed and the listerial burden in tissues evaluated by plating on blood agar. In one experiment we exposed pregnant mice (10 to 14 days gestation) to nitrite-supplemented drinking water before inoculation with *L. monocytogenes*as described above.

Results: Supplementation of mouse drinking water with $NaNO_2$ (25 or 50 mg/l) had no significant deleterious effect on resistance to listeriosis, as quantified by the recovery of viable *L. monocytogenes* from the spleen and liver at 3 days after inoculation. Nor did nitrite supplementation of drinking water (25 mg/l) have an adverse effect of resistance to listeriosis in pregnant mice. We recovered similar numbers of *L. monocytogenes*from the spleen, liver and fetoplacental units of pregnant mice given water nitrite-supplemented or control water.

Significance: Sodium nitrite supplementation of drinking water does not have an adverse effect on resistance of mice to gastrointestinal infection with *Listeria monocytogenes*. The results of this study provide additional information to inform the risk/benefit debate regarding nitrites in the diet.

P1-155 Comparing Cost of Illness and QALY Loss as Measures of Foodborne Illness Burden

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Introduction: Cost of Illness (CoI) and Quality Adjusted Life Year (QALY) measures are needed to assess and rank the public health significance of foodborne illness across pathogens or pathogen-food pairs. The National Academy of Sciences has recommended against combining these two measures, but little research has been conducted to examine the implications of this recommendation for food safety policy analysis.

Purpose: The purpose of this study is to assess empirical differences between CoI and QALY loss estimates and rankings across pathogens and pathogen-food pairs.

Methods: The study compares new QALY and CoI estimates and rankings for 14 foodborne pathogens and 168 pathogen-food pairs based on 2011 incidence estimates. It evaluates the influence of annual number of illnesses, hospitalizations and deaths on QALY and CoI rankings. It uses non-parametric statistical analysis to examine the relationships between rankings based on both integrated measures and on CDC incidence estimates. The study also examines the implications of data limitations on the completeness of QALY and CoI estimates and rankings.

Results: We find that existing research and data are sufficient to estimate both QALY loss and CoI due to acute illness with most foodborne pathogens, but that data on chronic sequelae are lacking for CoI. Despite this, we find QALY and CoI rankings to be highly correlated for pathogens, foods, and pathogen-food pairs. Both QALY and CoI rankings are driven by deaths and hospitalizations. Correlation between QALY and CoI measures falls considerably when focused on only the top 10-20 ranked pathogen-food pairs.

Significance: For overall rankings of all pathogen-food pairs, it makes little difference if rankings are based on CoI, QALY or deaths. As burden measures, CoI and QALYs each have meaningful strengths as well as empirical limitations. This study shows how these limitations affect estimates of the burden of foodborne disease in the U.S.

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P1-156 Chemical, Physical and Biological Indicators for *Salmonella* spp. in Central Florida Surface Waters

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Introduction: Coliforms or *Escherichia coli* levels are commonly monitored to determine microbial water quality; their usefulness as index organism for *Salmonella* is unknown. Chemical, physical and other biological parameters may be alternate indicators for *Salmonella* in surface water.

Purpose: The potential of biological (Aerobic Plate Count, *E. coli* and coliforms), chemical (pH, turbidity, conductivity and oxidation-reduction (OR) potential) and physical (water and air temperature) indicators to predict the levels of *Salmonella* in surface water was evaluated.

Methods: Samples were taken monthly for 12 months from 18 locations (216 samples). Air and water temperature, pH, OR potential, turbidity and conductivity were measured. Weather data was obtained from nearby weather stations. A three tube modified FDA-BAM *Salmonella* MPN was used to determine *Salmonella* levels. *Salmonella* isolates were confirmed using PCR for *invA* or *oriC* genes. Aerobic plate counts were determined by spread plating on tryptic soy agar; a three tube Colisure MPN was used for coliform/*E. coli* determination.

Results: Bivariate scatter plots of all indicator parameters and *Salmonella* levels were done. There were weak correlations ($r^2 \approx 0.1$) between *E. coli* and/or coliforms levels with *Salmonella* levels, suggesting that these biological indicators could not predict *Salmonella* levels. Similarly, chemical indicators could not predict ($r^2 < 0.1$) *Salmonella* levels. Scatter plots between biological and chemical indicators and biological and physical indicators did not indicate significant relationships. The average rainfall (previous month) before sampling did not correlate well with bacterial levels. Contour plots showed that most observations of *Salmonella*, *E. coli* and coliforms occurred at around 1, 2 and 4 log MPN/100 ml respectively.

Significance: The role of index microorganisms and chemical indicators in determining the prevalence of *Salmonella* in at least some surface water may be limited. In the absence of a rapid indicator, direct screening of surface water for*Salmonella* may be necessary where *Salmonella* levels are high and where waters are applied close to harvest.

P1-157 Risk Assessment of *Vibrio parahaemolyticus* in Fishery Products in Korea

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Introduction: Fishery products, which can be consumed without further cooking and/or reheating, can be considered potentially high-risk foods. Foodborne disease outbreaks associated with fishery products have generally been related to contamination by *V. parahaemolyticus*, as they are usually prepared by hand and stored at low temperatures. The most popular fishery products were monitored in this study, and a predictive growth model, frequency, consumption quantity and dose-response model were assessed in order to estimate the risk of *V. parahaemolyticus*contamination in fishery products in Korea.

Purpose: This study describes a quantitative microbial risk assessment (QMRA) model of Korean cases by *V. parahaemolyticus*due to the consumption of fishery products taking into consideration the primary data acquired during the exposure assessment step. The results of this QMRA can be used by administrators to establish national regulations for the control of foodborne diseases.

Methods: We developed a one-dimensional risk assessment model to describe mathematically the annual risk of *listeriosis*associated with the consumption of fishery products in Korea. We monitored microbial contamination levels of fishery products and frequency and consumption quantity for MRA.

Results: The mean and maximum number of cases by *V. parahaemolyticus*arising annually due to consumption of fishery product (flatfish sashimi) per fifty million individuals were estimated as 0.0165 and 3.38. These results indicated that the risk factors of regression sensitivities, from the retail-to-table pathway, could be applied to risk management. In the future, additional studies will be required to facilitate more realistic and accurate microbial risk assessments.

Significance: As the population of Korea numbers approximately 50 million, approximately 4 patients per year are expected to contract *V. parahaemolyticus* infections due to flatfish sashimi intake. Moreover, considering the increase in the consumption of fishery products, the actual risk is expected to be somewhat higher than the estimated risk. Also, additional studies regarding selective evaluation on groups of extreme intake and sensitive consumption, as well as the development of dose-response models.

P1-158 Prevalence, Antibiotic Susceptibility and Genetic Diversity of *Cronobacter* spp. from Desiccated Ready-to-Eat Products in Korea

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Introduction: *Cronobacter* spp. are considered to be an emerging opportunistic human pathogen and an etiological agent of life-threatening infections in infants. Symptoms include neonatal meningitis, sepsis and necrotizing enterocolitis, with a 40-80% mortality rate. Desiccated agriculture and marine products used as dry ingredients in PIF or baby food could be a dangerous source of *Cronobacter*infection in infants. Moreover, desiccated foods also can cause illness in elderly or immunocompromised adults, because some agricultural and marine desiccated foods are routinely consumed without further heat process in Korea.

Purpose: One hundred fourteen desiccated ready-to-eat food samples, including agricultural and marine products, were investigated for the presence of *Cronobacter* from January 2011 to January 2012.

Methods: Antibiotic resistance was assessed with the disk diffusion method, and the molecular subtypes of *Cronobacter*isolates were identified using an automated rep-PCR (repetitive sequence-based PCR) system.

Results: Thirteen *Cronobacter* strains (11.4%) were finally isolated from 114 desiccated Ready-to-Eat products. The most common antibiotic resistance of *Cronobacter* observed was against cephalothin (69.2%) followed by ampicllin (7.6%), but was susceptible to chloramphenicol (100%), ciprofloxacin (100%), gentamicin (100%), nalidixic acid (100%), tetracycline (100%), streptomocycin (100%). Also *Cronobacter* strains isolated from different sources were generally differentiated by using the automated rep-PCR system, indicating that it could be used for the purpose of contamination source tracking of the foodborne pathogen bacteria including *Cronobacter*spp.

Significance: The prevalence of foodborne pathogenic bacteria in desiccated ready-to-eat foods, and also the profiles of their antibiotic resistance determined in this study could be useful for the risk assessment of the potential infection of*Cronobacter*.

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P1-159 Risk of Infection with *Salmonella* and *Listeria monocytogenes* Due to Consumption of Ready-to-Eat Leafy Vegetables in Sao Paulo, Brazil

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Introduction: Although many quantitative microbiological risk assessment (QMRA) models have been developed in the last 15 years, few QMRA models for fresh ready-to-eat produce are currently available. The development of QMRA models focusing on fresh produce is very important because of increasing concern over fresh produce safety.

Purpose: The current study was carried out to estimate the risks of infection due to consumption of RTE vegetables contaminated with *Salmonella* and *Listeria monocytogenes*in Sao Paulo, Brazil.

Methods: The risk assessment model was composed of five different modules comprising the retail to consumption steps. A total of 8 scenarios were simulated using pathogen prevalence and concentration levels reported in RTE vegetables in Brazil as well as scenarios where lower prevalence and concentration were assumed. Scenarios where temperature during transportation and storage were maintained below 7°C were also evaluated. Models built in Excel spreadsheets were run (100,000 iterations) using @Risk software. The outputs obtained were risk of illness per month and predicted number of cases of illness in Sao Paulo, Brazil.

Results: The reduction of prevalence of *Salmonella* from 1.7% to 0.7% resulted in a decrease of risk of illness per month of up to 7 times. The reduction of prevalence of *L. monocytogenes* from 2.2% to 0.22% resulted in decrease of risk of infection from 1.87E-08 to 2.90E-09. The risks and number of cases predicted in scenarios in which temperature was kept below 7°C were significantly reduced for both pathogens when compared to scenarios using temperature data from the literature. The scenarios where prevalence and concentration of pathogens were reduced, and where temperature < 7°C led to the lowest number of cases of infection due by *Salmonella* and *L. monocytogenes*(185 and 3.81E-05 cases per month in Sao Paulo, respectively).

Significance: The results suggest that mitigation strategies that reduce pathogen prevalence and concentration as well as improved temperature control should reduce microbial risk from fresh produce in Sao Paulo, Brazil. More data are needed to improve the accuracy of risk assessment models developed.

P1-160 Potential Cross-contamination Transfer Dynamics at Retail Deli Markets

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Introduction: Ready-to-eat deli meat and poultry products are considered a food at high risk for causing foodborne illness. Cross-contamination in the retail deli may contribute to contamination of these products.

Purpose: Tracking potential cross contamination pathways is essential to reducing the risk of contaminating these products. This study tracked cross contamination through a retail deli using an abiotic surrogate, GloGerm™, to visually represent how pathogens may spread through the deli environment via direct contact on food contact surfaces.

Methods: The study examined six origination sites (i.e., slicer blade, meat chub, floor drain, preparation table, employee's glove, employee's hands) separately, where contamination may be introduced into the deli. Each site was inoculated with 20 mL of GloGerm™and a series of standard deli operations were completed (approximately 10 minutes of work). Photographs were taken to visualize spread of the contaminant throughout the deli. A sensory panel was used to evaluate the levels of contamination coverage and intensity on contaminated surfaces.

Results: Five of the six originating contaminated sites consistently spread contamination to the deli case door handle, slicer blade, meat chub, preparation table and the employee's gloves. Additional locations saw occasional contamination spread (i.e., deli case shelf, prep table sink, and glove box) but not consistently across all trials. There was no contamination spread from the floor drain to any food contact surfaces.

Significance: The findings of this study reinforce the need for consistent equipment cleaning and food safety practices among deli workers in order to minimize cross contamination.

P1-161 Microbiological Hazard Analysis on the Harvesting Steps of Perilla Leaf to Apply Good Agricultural Practices (GAP) System

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Introduction: Perilla leaf is one of the most consumed vegetable in the Republic of Korea. Direct consumption of this raw vegetable with improper washing may render the possibility of food poisoning outbreaks.

Purpose: This study validated microbial risk factors which may cause cross-contamination of food poisoning pathogens on the harvesting steps in perilla leaf farms.

Methods: Samples were collected from cultivation environments and utensils (soil and irrigation water), plants (perilla leaf and stem), personnel hygiene (hand, glove, cloth) and airborne bacteria at three farms (A, B, C) of perilla leaf on the harvesting steps. The collected samples were assessed for sanitary indications, fungi, and foodborne pathogens (*Staphylococcus aureus, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus,* and *Salmonella* ssp.).

Results: Total bacteria and coliform in perilla leaf were detected at the level of 4.4~5.2 and 3.4~4.3 log CFU/g, respectively. Meantime, *E. coli* was not detected in all samples. In case of pathogenic bacteria, *B. cereus* was detected at levels of 0.7 ~ 5.0 log CFU/g (or ml, hand, and 100 cm^2), and *S. aureus* was detected in worker's hand of farm A at levels of 3.4 log CFU/hand. Other pathogenic bacteria (*E. coli* O157, *L. monocytogenes and Salmonella* spp.) were not detected in either of the samples from all farms.

Significance: This study demonstrates that perilla leaf after harvesting was contaminated with various microbial risk factors. To minimize food poisoning outbreaks associated with perilla leaf, GAP system should be applied for ensuring the safety of perilla leaf.

P1-162 Development and Validation of a Predictive Growth Model for Pathogenic *Escherichia coli* O157:H7 in Red Leaf Lettuce

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Introduction: *Escherichia coli* O157:H7, an occasional contaminant of fresh produce, can present a serious health risk in minimally processed leafy green vegetables. To manage this human pathogen in leafy greens effectively, several predictive models for *E. coli* O157:H7 in leafy greens such as iceberg and romaine lettuce have developed. But predictive models that describe the behavior of *E. coli*O157:H7 in red leaf lettuce are lacking.

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Purpose: This experiment was conducted to develop an appropriate predictive model for QMRA (Quantitative Microbial Risk Assessment) on *E. coli* O157:H7 in red leaf lettuce.

Methods: First, growth curves of *E. coli*O157:H7 in lettuce were obtained at several isothermal conditions (5, 10, 15, 20, 25, 30 and 37°C) and were then fitted into Gompertz model with a high correlation coefficient.

Results: The resulting values for SGR and LT were fitted by quadratic and inverse second order equation, respectively. After that, verification of the developed models has been carried out using several mathematical or statistical indicators such as R^2, bias factor (Bf) and accuracy factor (Af).

Significance: We showed that R^2 values were close to 1, and Bf values of 0.84 and 1.03 and Af values of 1.27 and 1.24 were all in the acceptable range. This result demonstrated that overall predictions showed good agreement with the experimental values, indicating success at providing reliable predictions of *E. coli* O157:H7 growth in red leaf lettuce.

P1-163 Variability and Uncertainty Analysis of the Cross-contamination Rates of *Salmonella* during Pork Cutting

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Introduction: The transfer ratio of bacteria from one surface to another is often estimated from laboratory experiments and quantified by dividing the expected number of bacteria on the recipient surface by the expected number of bacteria on the donor surface. It can only be estimated with limited precision and its estimate may exceed 1 if real transfer is close to 100%. In addition, transferred fractions may vary over multiple experiments but it is unclear, using this approach, how to combine uncertainty and variability into one estimate for the transfer ratio.

Purpose: To develop a method by which uncertainty within one experiment is combined with variability over multiple experiments and by which inappropriate values for the transfer ratio are prevented.

Methods: A Bayesian Network model was developed for this purpose. The model was tested using data from a laboratory experiment in which the transfer of *Salmonella* from contaminated pork meat to a butcher's knife and from the knife back to pork meat was determined. Recovery efficiency of bacteria from both surfaces was also determined and accounted for in the analysis.

Results: The functionality of the model was demonstrated. The transfer ratio probability distributions were shown to have a large variability, with a mean value of 0.11 for the transfer of *Salmonella* from pork meat to the knife and 0.36 for the transfer of *Salmonella* from the knife to pork meat.

Significance: The proposed Bayesian model can be used for analyzing data from similar study designs in which uncertainty should be combined with variability.

P1-164 Microbiological Quality of Tilapia and Shrimp Ceviches and its Raw Material Sold at the Metropolitan Area of San Jose, Costa Rica

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Introduction: Ceviche is a commonly used dish from Latin American countries, made up of raw fish or seafood and marinated with lemon or lime juice, added with several seasoning ingredients. Its manufacturing includes the normal flora coming from raw product but also acquired flora, coming from manufacturing duties and additional ingredients. Also, it is a product that is consumed raw, representing a risk for public health.

Purpose: The aim of this work was to determine the microbiological quality of tilapia ceviche and shrimp ceviche sold at the metropolitan area of San José, Costa Rica, as well as the raw material used in its elaboration.

Methods: 25 samples of tilapia ceviche and 27 samples of shrimp ceviche were analyzed; same number of raw material was analyzed. Also, the behavior of some of the spoilage populations was analyzed in 25 samples of shrimp and same number of tilapia ceviche during its storage at 4°C. Analysis included the evaluation of indicators and of different potential pathogens including *Listeria monocytogenes, Salmonella, Escherichia coli, Vibrio parahaemolyticus* and *V. cholera*.

Results: The results obtained from the raw material and ceviche analysis show that, although there is an important bacterial contamination present in raw material, and that the ceviche is a product that includes manipulation, there is an important reduction in the bacterial charges present in the end product, especially referring to total aerobic psychrofilic plate count and total coliforms; but not to fecal coliforms. *Escherichia coli* was isolated from 15% of the tilapia's ceviche and 4% from shrimp's ceviche, 3 strains of *Listeria monocytogenes* were isolated from tilapia, one from shrimp, 3 strains of *V. parahaemolyticus* were isolated from shrimp and 2 from its ceviche. *Salmonella* spp. were not isolated. The pH of ceviche plates stored at 4°C for one week showed a trend towards stability or slight increase of this parameter. Same way, the concentration of spoilage microorganisms suffered slight variations; nevertheless, the low number of *Pseudomonas* spp present in tilapia's ceviche is an outstanding result since this population is associated to spoilage of the product.

Significance: Although there is an important reduction in the number of bacteria present in ceviches compared to raw material, the isolation of potential pathogens show that these products may represent a risk for public health.

P1-165 Comparison between the Exponential and Weibull-gamma Dose-response Model Approaches to Quantitative Microbial Risk Assessment

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Introduction: Quantitative microbial risk assessment (QMRA) can be to provide scientific advice to the risk managers who will use the information to decide upon the risk management option(s) that will be implemented to achieve the desired level of consumer protection from microbial hazards. According to the Codex Alimentarius Commission, a QMRA should include four steps, i.e., hazard identification, exposure assessment, hazard characterization, and risk characterization. In these above successive steps, a crucial aspect of QMRA is the assessment of dose-response relationship since they are critical to the finally risk evaluation and also the selection of this model had a significant impact on the magnitude of risk estimates.

Purpose: The objective of this study was to compare two dose-response models, i.e., exponential and Weibull-gamma, and determine which model was more appropriate for QMRA of *Listeria monocytogenes*in ham products in Korea.

Methods: The model was constructed for QMRA of *L. monocytogenes* with consumption on ham products in Korea, according to Codex guidelines. Frame-work model as product-retail-consumption pathway composed with initial contamination level, the time and temperature in distributions, and consumption data sets for ham products and also used the developed predictive growth. To determine more appropriate of

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two dose-response models QMRA of *L. monocytogenes*used and compared the exponential and Weibull-gamma model. The simulation model and formulas with Microsoft®Excel spreadsheet program using these data sets was developed and simulated with @RISK.

Results: The probability of foodborne disease by *L. monocytogenes* with consumption of the ham products per person per day, in which simulated with the exponential and Weibull-gamma dose-response models, was estimated similarly as 5.80×10^{-14} , 3.71×10^{-14} in low risk population, and 2.80×10^{-10} , 7.07×10^{-10}in high risk population, as a mean values, respectively. However, the lesser estimated risk level, the Weibull-gamma model greater risks than the exponential model with Pearson correlation coefficients value was 0.8009.

Significance: This study can be used as scientific information and showed the practical possibility using and picking up dose-response models for QMRA.

P1-166 Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 Inoculated in Raw Peanuts Stored at -20, 4, and 23°C

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Introduction: Peanuts can become contaminated with foodborne pathogens during growth, harvest and storage and the survival of the pathogens on peanuts may depend on the storage conditions. Few reports have focused on how storage temperatures impact the survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on raw peanuts.

Purpose: This study evaluated the survival of *Salmonella* spp. and *E. coli* O157:H7 on the surface of raw peanuts inoculated at different levels and stored at various temperatures.

Methods: Raw peanuts were inoculated with three strains of *Salmonella* and three strains of *E. coli* O157:H7 which were associated with foodborne outbreaks. For each pathogen, the peanuts were inoculated at three levels (ca. 6, 4 and 2 log CFU/g) and stored under three temperature conditions (room temp, 23 ± 3°C; refrigerated, 4 ± 2°C; and frozen, -20 ± 2°C) for three months. Triplicate samples of 100 g each were pulled at three week intervals and analyzed using selective media (XLD for *Salmonella* and CTSMAC for *E. coli* O157:H7).

Results: Both *Salmonella* and *E. coli* O157:H7 levels declined rapidly in room temperature raw peanuts, but more slowly when the peanuts were refrigerated or frozen. The average reduction rates across three levels of *Salmonella* inoculum (± standard deviation) for room, refrigerated and frozen temperatures were -0.72 ± 0.20, -0.09 ± 0.07 and -0.10 ± 0.05 log CFU/month, respectively; while the averaged rates for three levels of *E. coli* O157:H7 inoculum for room, refrigerated and frozen temperatures were -0.63 ± 0.27, -0.16 ± 0.07, -0.12 ± 0.07 log CFU/month, respectively.

Significance: The information developed in this study can be used in risk assessment modeling to account for reductions which might occur during the production of peanuts products when stored at various temperatures, in distribution and after purchase by consumers.

P1-167 An Alternative Approach for Predicting Probability of Pathogen Growth on Iceberg Lettuce Using Logistic Regression

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Introduction: Bacterial pathogens such as *Escherichia coli*O157:H7 can infect with low dose ingestion such as < 100 CFU/g. Conventional predictive models that intend to describe entire growth kinetics do not directly evaluate the risk of infection. In order to evaluate the risk of infection of bacterial pathogen, probabilistic model that can directly predict small amount of growth will be useful.

Purpose: The objective of this study was to develop a probabilistic model to predict the probability of the time to 1-log increase of *E. coli* O157:H7, *Salomonella* spp., and *Listeria monocytogenes*on iceberg lettuce during chilled to room temperature storage.

Methods: Changes in the cell number of *E. coli* O157:H7, *Salomonella* spp., and *L. monocytogenes* on fresh-cut iceberg lettuce was evaluated between 5 to 25°C. The time for 1-log increase from the initial cell number was calculated from the obtained growth kinetics. The whole kinetic data was evaluated whether 1-log increase (1) or not (0) on each sampling interval. The evaluated data was modeled using logistic regression procedure as a function of temperature, time, and kind of bacteria.

Results: The probability of time to 1-log increase was successfully modeled for each bacterium using logistic regression with high accuracy (percent concordant; 85.9%, AIC; 65.7). Furthermore, we obtained the probability density distribution by differentiation of the obtained probability model. This function enabled to calculate the probability of 1-log increase within arbitrary periods of storage time.

Significance: The developed model allowed us to estimate not only the probability of time to 1-log increase of each pathogen on iceberg lettuce but also its probability density distribution. The model developed in this study can be used to evaluate the infection risk of each pathogen in conjunction with those dose-response models.

P1-168 Prevalence of *Salmonella* and *Escherichia coli* O157:H7 in Cow/Calf Operations in Texas, New Mexico and Oklahoma during the Summer and Fall Months

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Introduction: Cattle are a common reservoir for pathogens such as *Salmonella* and *Escherichia coli* O157:H7. In past years, a number of prevalence studies have been conducted in dairy and feedlot operations but prevalence data in the cow/calf sector is scarce.

Purpose: The purpose of this study is to help understand prevalence in the cow/calf operations in the Southwest U.S.

Methods: Thirty cow/calf pastures were investigated during this experiment. From each cow/calf pasture fifteen fecal samples, three water, three swab and three sediment samples were tested for *Salmonella* and *Escherichia coli* O157:H7. Samples were collected from most ranches twice in the Summer and Fall months of 2011. Sampling supplies were shipped from Texas Tech University to cow/calf operations with instructions to obtain samples and shipped back in cold conditions to TTU for microbial analysis. *Salmonella* and *E. coli* O157:H7 were detected in water samples using PCR analysis with the BAX system. Fecal and sediment samples were tested for *Salmonella* presence by enriching samples in RV and TT for 24 hours at 42°C and plating on XLT-4. Fecal and sediment samples were tested for *E. coli* O157:H7 using IMS protocols and biochemical confirmation.

Results: During summer sampling, fecal samples were 4.8% positive for *E. coli* O157:H7 (n=900) and 5.8% positive (n=450) for *Salmonella. Salmonella* and *E. coli* O157:H7 were not detected in water samples in the fall or summer. *E. coli*O157:H7 was not detected in any swab sample and only 1.1% of swab samples (n=90) were positive for *Salmonella*. Sediment samples were 2.2% (n=90) positive for *Salmonella* and 0.002% positive for *E. coli* O157:H7 (n=180).

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During fall sampling time, fecal samples were 1.1% positive (n=900) for *E. coli* O157:H7 and 3.33% (n=450) positive for *Salmonella.* Swab samples were all negative for both pathogens. Sediment samples were 1.1% positive for *Salmonella* and 0% positive for *E. coli* O157:H7.

Significance: While the prevalence of pathogens were low in cow/calf operations in the Southwest, more data are needed over various years and different geographical locations to gain more understanding of the behavior of pathogens in these environments.

P1-169 Considering Uncertainty and Variability in Models for Assessing the Microbiological Shelf-life of Foods

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Introduction: Integrating variability and uncertainty in predictive modeling clearly improves the reliability of Quantitative Risk Assessment (QRA), and fulfills with all the stakeholders expectations: regulation bodies, food operators, quality managers. The same approach can be used to assess as well the food product shelf-lives.

Purpose: The impact of both, variability and uncertainty was quantified in the microbiological shelf-life estimation of food products.

Methods: Various growth kinetics, within specific food items, were modeled, using the same environmental conditions of temperature, pH and water activity. The study was thus run with *Listeria monocytogenes* as bacterial model. Three products were studied: pâté, cured herring and smoked chicken. The numbers of growth kinetics (n=3; 10), dates (n=5; 10) and points (n=1; 3) were considered to evaluate the variability and uncertainty related to modeling. The estimation of growth parameters were characterized with a Non-Linear Mixed Effect models, based on the stochastic version of Expectation-Maximisation Algorithm (SAEM). These estimates were obtained with the Monolix software, and were then used for simulation with the 2D Monte Carlo calculation in the Sym'Previus decision making tool (www.symprevius.org).

Results: The proposed stochastic approach calculates the probability to overpass with precision a critical value during product shelf-life. While the demonstration was done with *L. monocytogenes*species, the same calculations can be applied to predict the quality indicators and spoilage bacteria behavior.

Significance: Variability and uncertainty in the initial contaminations and in the chemical parameters, such as pH and water activity, could be integrated in the developed stochastic model to determine the probability to exceed microbial criteria during food storage. The approach is particularly useful to estimate food product shelf-lives, to run QRA, to determine and target the significant microbial and chemical quality controls in HACCP.

P1-170 Impact of Product Water Activity on the Validity of Thermal Inactivation Models for *Salmonella* on Almonds

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Introduction: Food processors still have limited means to determine which pasteurization validation method for *Salmonella* lethality yields the most accurate or reliable result for low-moisture foods. Water activity (a_w) affects *Salmonella*thermal resistance; however, the relative effect of process humidity and product a_w on inactivation rates has not been quantified.

Purpose: The purpose of this study was to evaluate whether product a_w significantly affects the validity of five different process validation methods for *Salmonella*inactivation on almonds.

Methods: Almonds were inoculated with *Salmonella* Enteritidis PT30 or *Enterococcus faecium* (NRRL B-2354) as a *Salmonella* surrogate, at ~10^8 CFU/g and equilibrated to 0.45 or 0.60 a_w, and then heated in a pilot-scale moist-air impingement oven (dry bulb 121, 149, or 177 °C; dew point < 33, 69.4, 81.6, or 90.6°C; v_{air} = 2.7 m/s) to a target lethality of 4 log. Surviving *Enterococcus* and *Salmonella* were enumerated (3 reps per treatment) by plating on deMan, Rogosa and Sharpe agar or trypticase soy agar modified with sodium thiosulfate and ammonium iron citrate (35°C, 48 h), respectively. Almond surface temperatures were measured (9 reps per treatment) using surface thermocouples (T_{surf}) on almonds and aluminum almonds (T_{Al}), with these temperatures then used to calculate *Salmonella*inactivation using a traditional (D, z) model and a previously published modified model accounting for process humidity.

Results: Among all process validation methods, *E. faecium* yielded the lowest root mean squared error in predicting *Salmonella* inactivation (RMSE = 1.03 log (CFU/g), n = 12, a_w = 0.45). For the same data, the modified model yielded an RMSE of 2.08, and the traditional model exhibited unacceptably high error (RMSE > 16). Even with the modified model accounting for process humidity, a_w significantly affected model accuracy, with the RMSE doubling between 0.45 and 0.65 a_w for the model developed with low a_w data.

Significance: Overall, product a_w is a critical factor that affects the accuracy of process validation methods. Therefore, thermal inactivation models for moist-heat conditions ideally should account for both process humidity and product a_w.

P1-171 Time Temperature Pathogen Predictor (T2P2): Expansion of a Risk Assessment Tool Based on Interval Accumulation of Dynamic Temperature Profiles Associated with Short-term Temperature Abuse of Raw Shrimp

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Introduction: Pathogenic bacterial growth and toxin formation on shrimp can result from short-term temperature abuse and subsequently cause illness from consuming raw, undercooked or re-contaminated shrimp. Pathogens can be introduced during harvest, handling, or processing and further growth can occur during storage and transportation. Information related to short-term temperature abuse of *Vibrio parahaemolyticus* (VP), *Salmonella* sp. (SAL), and *Staphylococcus aureus*(SA) is needed to mitigate the risks associated with these hazards and to support process deviation decision making.

Purpose: The purpose of this study is to evaluate the lag phase duration (LPD) and the growth rate (GR,) of VP, SAL and SA at a variety of times and temperatures during short-term temperature abuse in a "real" food matrix and thereby expand the database of the T2P2 predictive modeling tool.

Methods: Three separate cocktails (five strains each) of VP, SAL and SA were prepared and inoculated separately into ground shrimp. The final concentration was approximately 10^4/g in 25.0 g of shrimp. The experimental temperature range was 10 – 43.3°C at 10°C increments. Samples were withdrawn at various time intervals at each incremental temperature, diluted and plated on appropriate media and incubated. Data obtained as CFU/ml were fitted with the DMFit 2.0 program which generated LPD and GR values.

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Results: For all organisms, LPD decreased and GR increased as the temperature increased from 15.6 – 43.3°C. For example, the LPD for VP decreased ~ 12 fold, from 565 to 46 min, and the GR increased ~ 6 fold, from 0.004 to 0.023 {log (CFU/ml/min}. The comparative difference in GR of VP, SAL, and SA at 43.3°C was 0.023, 0.015 and 0.015 {log (CFU/ml/min}, respectively.

Significance: The database expansion for shrimp is nearly completed and validation studies are in progress, as is work with Risk Sciences International (software developer) to further develop the T2P2 web-portal and User Guide to aid in the food safety risk decision making process.

P1-172 Impact of the Local Microenvironment in a Food Matrix on *Salmonella* Survival

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Introduction: Multiphasicfoods can be problematic in process validations. Ingredients can create local microenvironments that may determine process effectiveness and be more critical to pathogen survival than the overall physicochemical properties of the food. Although it has been speculated that local microenvironments can affect pathogen survival, experimental validation of this concept has not been performed.

Purpose: To study the impact of the local microenvironments on the survival of *Salmonella* in model multiphasicfood systems.

Methods: Non-fat dry milk powder (NFDM) and creamy peanut butter (PB) were combined to create model multiphasic systems having identical compositions but different physicochemical local microenvironments. *Salmonella* cells were mixed into either matrix, and then combined with the other in different sequential order, as follows: 10 log CFU *Salmonella* cells in 0.5 ml of PBS was added to 5 g NFDM or 20 g PB, homogenized, and then mixed into 20 g PB and 5 g NFDM, respectively. Cells were also inoculated into 25 g pure PB and NFDM, respectively, as control systems. Viable cells were enumerated by plate counting within 1 h of inoculation and after 5 weeks of storage at 25°C. The distribution of water, lipid, and protein in the microenvironment was analyzed by micro-FT-IR using a mid-IR ATR image analysis system. Three independent experiments were carried out using duplicate samples in each. Data were analyzed using One-Way ANOVA andTukeyHSD test.

Results: Each inoculated model system showed a statistically uniform distribution in terms of bacterial counts per gram, but not homogenous absorptions of amines, OH, C-C, and C=C groups, as determined by FT-IR imaging with chemical specificity and high spatial resolution analysis. Within one hour of inoculation, the log reduction was about 4-fold greater ($P < 0.05$) in the system with NFDM (2-log reduction) as the first ingredient than the one in which PB was first (0.55-log reduction). This log reduction pattern was consistent between the pure NFDM (2.5-log reduction) and pure PB (0.67-log reduction) ($P < 0.01$). After 5 weeks of equilibration, a 2-fold greater log reduction ($P < 0.01$) was observed in the system with NFDM (4-log reduction) as the first or only ingredient than the one in which PB was in immediate contact with cells (2-log reduction).

Significance: This study is the first report which provides evidence that characterized the local microenvironment and its role on *Salmonella* survival. Pathogen survival and process effectiveness may depend on whether the contaminated ingredient is miscible or remains as a discrete phase within the food.

P1-173 Predictive Microbiology Approach for Enumeration of *Salmonella* on Chicken Parts during Pre-enrichment

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Introduction: A data gap identified in risk assessments for *Salmonella* and chicken is lack of quantitative data. Enumeration of *Salmonella* on chicken parts is difficult because *Salmonella* are often present in low numbers. However, during the pre-enrichment phase of *Salmonella* isolation from chicken parts there is a mathematical relationship between the initial number of *Salmonella* on the chicken part and the number of *Salmonella*in the pre-enrichment broth at early times of pre-enrichment.

Purpose: Therefore, the current study was undertaken to develop a predictive microbiology approach for enumerating *Salmonella* on chicken parts during pre-enrichment.

Methods: A sterile cutting board and knife were used to partition a whole chicken into two wings, two breasts, two drumsticks and two thighs. A sterile, cooked chicken breast was then cut into two equal-sized portions using the cutting board and knife used to partition the whole raw chicken; this was done to study transfer of *Salmonella* from raw chicken to cooked chicken during meal preparation. Chicken parts were pre-enriched in 400 ml of buffered peptone water (BPW) for 6 h at 42°C and 80 rpm. For predictive model development, chicken parts were inoculated with 0.36 to 4.86 logs of *Salmonella*. At 6 h of incubation, the concentration of *Salmonella* in the BPW pre-enrichment was determined by spiral plating onto XLT4 agar media. A two-phase linear model was used to model the concentration of *Salmonella* in BPW as a function of the log number of *Salmonella*inoculated.

Results: All standard curves had high goodness-of-fit (R^2 from 0.92 to 0.99) to the two-phase linear model regardless of the serotype ($n = 4$) of *Salmonella* used. Prevalence of *Salmonella*among chicken parts was 2.84% (5/176). The positive chicken parts were thigh from chicken #4, which was contaminated with 3 cells of serotype Kentucky, and both wings, one thigh and one cooked breast portion from chicken #15, which were all contaminated with 1 cell of serotype 8,20:-:z6.

Significance: These results indicate that a predictive microbiology approach can be used to enumerate low numbers of *Salmonella* on chicken parts during pre-enrichment. However, because of the low prevalence of *Salmonella* on the chicken parts examined it was not possible, at this time, to fill the data gap for enumeration data identified in recent risk assessments for *Salmonella* and chicken.

P1-174 A Quantitative Meta-analysis of Existing Foodborne Pathogen Transfer Data

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Introduction: Bacterial cross-contamination between various food products and surfaces has been a major ongoing problem leading to numerous outbreaks and recalls. The number of papers published on bacterial transfer to/from food has increased approximately ten-fold over the past two decades, reflecting an increasing attention to this important issue. However, there has been no standardization of methods or aggregation of data in this field.

Purpose: Therefore, the objective was to conduct a quantitative meta-analysis of the published literature from the past ~40 years on transfer of foodborne pathogens, in order to evaluate the characteristics of the existing data, critical gaps, and the opportunity to aggregate these data for future analyses and modeling.

Methods: Data sources were collected initially via a keyword search in the ISI Web of Science database, to identify all studies including transfer data for key foodborne pathogens (*Listeria*, *Salmonella*, *Escherichia coli*, and *Campylobacter*). Published transfer data were characterized in terms of pathogen, product type, surface, and other variables.

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Results: The total analysis yielded ~55 distinct publications on foodborne pathogen transfer, of which 43 contained numerical data, with a total of 756 data sets, ~1,194 individual replicate curves, and over 14,456 individual observations quantifying pathogen transfer between food products and contact surfaces, including: meat/poultry (n = 29) and produce (n = 11), with transfer to metals (n = 19), plastics (n = 16), hands/gloves (n =10), and several other surfaces. These studies included a wide variety of other variables, such as contact time (n = 10), temperature (n = 12), inoculation level (n = 6), inoculated surface (n = 7), material composition (n = 9), and surface roughness (n = 4). These papers have been organized into a preliminary database defining the characteristics of each study, which will serve as the foundation for a broader, publicly-available database in this domain.

Significance: A unified database that aggregates pathogen transfer data, and becomes a repository for future data, will help advance linkages between fundamental research and the observed transfer outcomes, while also improving the design of future studies to fill critical data gaps.

P1-175 Evaluation of Norovirus Dose-response Models with Outbreak Data

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Developing Scientist Competitor

Introduction: Quantitative Microbial Risk Assessment (QMRA) of norovirus exposures is limited by the fact that available dose-response data are for strains that are no longer circulating. However, norovirus gastroenteritis outbreaks associated with the consumption of raw oysters are quite common. Outbreak reports for which detailed exposure factors and health outcomes are well-reported provide a unique opportunity to evaluate dose-response relationships for circulating strains of norovirus.

Purpose: The objective of this study was to develop a QMRA of consumption of raw oysters contaminated with norovirus, to populate the assessment with data from well-characterized outbreaks, and evaluate the dose-response relationship for circulating strains of norovirus.

Methods: A 2D Monte Carlo-based exposure model was developed in Crystal Ball (Oracle Corp., Redwood Shores, CA) and used to simulate dose of norovirus per serving of raw oysters in well-characterized outbreaks. Dose was modeled based on published concentration and occurrence of virus in the oysters, serving size, and an estimate of percentage of particles that are infectious (10 to 100%). Previously characterized dose-response models were then used to estimate the probability of illness. Predicted illness rates were compared to actual reported illness rates for the reported outbreak conditions.

Results: The exposure module estimated dose levels in the range of 25,000 to 43,000 copies of norovirus per serving of oysters. At these doses, the estimated probability of infection ranged from 0.50 to 0.63 depending on the dose-response model. The results demonstrate that the dose-response models provide estimates consistent with observed attack rates (46 and 67%) in examined outbreaks.

Significance: The modeled dose range (25,000 to 43,000 copies) for the examined outbreaks is in a region where the previously proposed dose-response models converge. Additional outbreak analysis or clinical studies are needed in the lower range of doses where the dose-response models diverge to further evaluate the models for circulating strains of norovirus.

P1-176 Modeling the Effect of Temperature and pH on the Lag Time of *Salmonella* on Cut Tomatoes

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Introduction: Outbreaks of salmonellosis associated with fresh cut tomatoes has been a food safety concern recently. When tomatoes are cut, *Salmonella* can be transferred from tomato skin to flesh, which is an amicable growth environment.

Purpose: The manipulation of pH and incubation temperature offers one possible means for *Salmonella* control in cut tomato products. With the regression model of growth rate with pH and temperature variables built first, the purpose of this research was to expand our existing research efforts on modeling *Salmonella* in fresh cut tomatoes, resulting in regression models able to predict both growth rate and lag time of *Salmonella* as a function of both pH and temperature.

Methods: Whole red round tomatoes were dip-inoculated in a cocktail of *Salmonella* strains obtained from the CDC. These strains were human isolates from cases associated with prior tomato salmonellosis outbreaks. Inoculated tomatoes were dried, cut into slices and incubated at temperatures from 10 to 30°C in 5-degree intervals. The pH of the cut tomatoes was adjusted from 3.8 to 4.2 by adding 5% citric acid. Samples were enumerated by plate counts on XLT4 agar until*Salmonella* populations reached stationary phase. Growth rates were calculated by DMfit software; lag time is calculated in Excel.

Results: A plot of square root (SQRT) of the growth rate (GR) of *Salmonella* under various temperature and pH=4.0 was linear with time, such that SQRT(GR)= 0.022T+1.148 (R2=0.79), which is slower than the growth rate under tomato's natural pH (~pH=4.4) found in previous studies. Moreover, *Salmonella*growth was suppressed at pH=3.8 at all temperatures from 10 to 30°C.

Significance: The models of *Salmonella* in cut tomatoes built in this project provide useful tools of estimating the risk by growth rate and lag time posed by different temperature abuse and pH manipulation.

P1-177 Willingness to Pay for HACCP in Foodservice

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Introduction: Implementation of Hazard Analysis Critical Control Point (HACCP) systems is voluntary in commercial retail foodservice organizations like restaurants, grocery store buffets, and gas stations/convenience stores. Unless management understands and is aware of benefits associated with HACCP implementation, profit motives could discourage voluntary participation in such businesses.

Purpose: This research examines the potential benefits (increased prices) to restaurants for improving product quality (in this case, food safety) by assessing consumers' willingness to pay (WTP) for such higher quality foods. The purpose was to investigate whether consumers would pay a price premium for HACCP in a foodservice establishment.

Methods: Consumers' willingness to pay was examined through a choice experiment conducted in a real-life restaurant setting. Consumers were provided an endowment for the experiment, and they could keep the unspent balance. This research design motivated participants to reveal their true preferences for safer foods. The sample of 880 individuals was diverse, and included the local community, rather than restricted to students or university staff/faculty. Data was analyzed using discrete and continuous regression methods.

Results: This study found that consumers were willing to pay a price premium of up to 32% on the base price. The findings also suggest that women consistently chose the HACCP-approved meal more than men over the alternative, and were willing to pay a higher price than did men –

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though this later finding was not statistically significant at $P < 0.10$. Results also characterize consumer meal choices and WTP based on HACCP information provided to the participants, type of service (buffet versus sit-down), amount of the endowment, and meal preferences (meat, poultry, vegetarian, seafood).

Significance: This study fills a critical gap in understanding WTP for HACCP in foodservice establishments. It also provides such establishments with an understanding of benefits for voluntarily implementing HACCP.

P1-178 Implementation of Food Safety Management Systems in Small Enterprises in Cyprus

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Introduction: EU legislation requires all food businesses to implement a food safety management system based on HACCP principles. Although manufacturers have used this system successfully for many years, it has been less common in small and medium sized enterprises (SMEs), especially those in the food service sector.

Purpose: When Cyprus joined the European Union in 2004, all food businesses had to comply with EU legislation. Many SME's in Cyprus had no food safety management systems in place at this time so a longitudinal study was set up to assess the impact of these systems on the hygiene of the businesses. The study also identified barriers to implementation in the study group.

Methods: An investigation was carried out from October 2005 to April 2008 using a sample of 50 SMEs located in the island of Cyprus. The study assessed the implementation of HACCP, CYS 244 and ISO22000 by measuring specific hygiene indicators. These were assessed at five critical points in the process and included microbiological standards, environmental contamination, hygiene practises, knowledge and attitude, and cost of implementation.

Results: The results show that initial implementation of Pre-Requisite Programmes and a simplified HACCP plan resulted in a significant improvement ($P < 0.05$) in all the parameters measured. However, most particpants encountered problems in applying and maintaining the systems and each enterprise had its own application limit regarding the complexity of the system. When this limit was exceeded, negative results appeared for the enterprise, indicating deterioration in hygiene. For some parameters, e.g., microbiological standards, the final measurements, after implementation of the most complex system, indicate more failures (10.6% of samples) than the baseline measurements taken before implementation of any system (8.4%).

Significance: The results suggest that while aspects of food safety management such as PRP's can help improve premises hygiene, attempting to implement a food safety management system that is too complex for an SME can result in the deterioration of food hygiene in the premises according to certain food safety indicators.

P2-01 Monitoring of *Salmonella* spp. on Egg and Liquid Whole Eggs from Egg-breaking Plant in Korea

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Introduction: Poultry products and eggs are the major cause of *Salmonella* food poisoning. Because there were many illnesses by *Salmonella* Enteritidis from contaminated eggs, it is necessary to monitor eggs and egg products.

Purpose: Since there were no specific data on *Salmonella* prevalence on egg products in Korea, we investigated the *Salmonella* contamination of eggs and liquid whole eggs from an egg-breaking plant.

Methods: We collected 800 eggs, and 40 unpasteurized and 30 pasteurized liquid whole egg samples from eight egg-breaking plants in spring, summer and winter during 2011. Samples were incubated with 225 ml buffer peptone water (BPW) solution by adding 25 ml shell egg pools (20 eggs), 25 ml unpasteurized and pasteurized whole liquid eggs. After 1 ml and 0.1 ml incubated BPW solutions were transferred 10 ml tetrathionate broth and 10 ml rappaport vassiliadis broth, respectively, they were incubated at 36 °C, 42 °C during 24 hours. The two incubated solutions were streaked on xylose lysine desoxycholate (XLD) agar. Colonies suspected to be *Salmonella* were confirmed by PCR and VITEK. The gene diversity of isolates was investigated by Rep-PCR.

Results: *Salmonella* weren't detected on eggs, unpasteurized and pasteurized liquid whole eggs collected in spring and winter. In contrast, *Salmonella* were detected in four unpasteurized liquid whole eggs from 2 egg-breaking plants (A and B) and *Salmonella* were detected in five pasteurized liquid whole eggs from only one egg-breaking plant (A) collected in summer. Seven of *Salmonella* isolates from A plant were all *S.* Bareilly which showed above 95% similarity. *S.* Bareilly from feces of farm that supplied eggs to plant was isolated and they showed 95% similarity with it from liquid whole egg. Two of *Salmonella* isolates from B plant were *S.* Bareilly and *S.* Richmond.

Significance: *Salmonella* spp. were isolated for the first time from pasteurized and unpasteurized liquid whole egg collected in summer two egg-breaking plants in Korea.

P2-02 *Listeria* spp. and *Listeria monocytogenes* in Beef Cuts and in a Beef Processing Plant Located at Minas Gerais State, Brazil

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Introduction: *Listeria monocytogenes* is a foodborne pathogen frequently associated with beef and meat products, usually due to the utilization of several types of equipment and utensils during the processing. Thus, the environment of beef processing facilities can play an important role in the contamination route of this pathogen.

Purpose: Verify the occurrence of *Listeria* spp. and *L. monocytogenes* in end products (beef cuts) and in the environment of a beef processing facility.

Methods: A beef processing facility was selected for the present study, where the following 222 samples were collected by swabbing (400 cm^2): shoulder (24 samples), tenderloin (24), rump (24), tables, knifes and workers hand before (11, 31, and 33, respectively), and during (11, 31, and 33, respectively) processing. All samples were subjected to *Listeria* spp. detection by ISO 11290-1, being the isolates submitted to biochemical identification.

Results: *Listeria* spp. was detected in 50 (22.5%) samples, being more frequent in tables and hands during processing (11/33 and 7/31, respectively) and shoulder (15/24). Based on biochemical results, 142 isolates were identified as *Listeria*spp., being 111 *L. innocua*, 26 *L. monocytogenes*, and 5 *L. grayi*. *L. innocua* was present in all samples at different frequencies, and *L. grayi* was detected only in tables during processing (3/33) and shoulder (1/24). *L. monocytogenes*, the main concern in this genus, was detected in knife before processing (1/11), hands and tables during processing (1/31 and 1/33, respectively), rump (1/24) and shoulder (5/24).

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Significance: These data indicate that *Listeria* spp. and *L. monocytogenes* are present in the beef cuts and the processing environment of the analyzed facility, showing the shoulder as the cut with the highest frequency of *L. monocytogenes*. Further analyses are necessary to determine the molecular profiles of isolates, in order to establish their persistence in this environment and their contamination routes.

P2-03 PFGE Characterization and Adhesion Capability of *Listeria monocytogenes* Isolates Obtained from Bovine Carcasses and Beef Processing Facilities

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Introduction: *Listeria monocytogenes* is a pathogen capable of adhering to many surfaces and forming biofilms, which may explains its persistence in food processing environments, especially beef processing facilities. In addition, this pathogen is relatively resistant to variations in pH, NaCl and several antimicrobial substances, facilitating its permanence in the environment.

Purpose: This study aimed to genetically characterize *L. monocytogenes* isolates obtained from bovine carcasses and beef processing facilities and to evaluate their adhesion capabilities.

Methods: DNA from twenty-nine *L. monocytogenes* isolates was subjected to enzymatic restriction digestion using *Asc*I and *Apa*I. All isolates were evaluated for the adhesion capability in microtitre plates considering the following variables: inoculum concentration, culture media, carbohydrate source, NaCl concentration, incubation temperature, and pH. **Results** were compared by ANOVA and Tukey ($P < 0.05$).

Results: Two clusters were identified for serotypes 4b and 1/2a, with similarities of 48% and 68%, respectively. The isolates presented best adhesion performance when tested at 8 log CFU/ml, being classified according to its capability as weak (8 isolates), moderate (17) or strong (4). The isolates showed higher adhesion capability in non-diluted culture media, medium at pH 7.0, incubation at 25 °C and 37 °C, and medium with NaCl concentrations of 5% and 7%. No relevant differences were observed for adhesion capability with respect to the carbohydrate source ($P > 0.05$).

Significance: The results indicated that despite a wide variation of characterized PFGE profiles, *L. monocytognes* adhesion might be related to optimal growing conditions.

P2-04 Monitoring of Hygiene Indicator Microorganisms in Bovine Carcasses from Three Slaughterhouses Located in Minas Gerais State, Brazil

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Introduction: The different steps of bovine slaughtering represent important sources of microbiological contamination of carcasses and meat products. For this reason, constant monitoring of these steps is crucial in order to guarantee the international standards of quality and safety of beef products.

Purpose: This study aimed to evaluate the microbiological contamination in different points of the bovine slaughtering, in order to compare the hygienic profiles from three slaughterhouses located at Minas Gerais State, Brazil.

Methods: Three slaughterhouses (Sl.1, Sl.2, and Sl.3, all inspected by the Brazilian Federal Inspection Service) were selected. From each one, 65 bovine carcasses were sampled by swabbing (400 cm²) in four distinct steps of the slaughtering process (A: bleeding, B: after skinning, C: after evisceration, and D: after last washing). All samples were submitted to analyses to enumerate mesophilic aerobes (Petrifilm™ AC), Enterobacteriaceae (Petrifilm™ EB), coliforms and *Escherichia coli*(Petrifilm™ EC). The obtained counts (log CFU/cm²) were compared by ANOVA and Tukey ($P < 0.05$) to verify significant differences between slaughtering steps and slaughterhouses.

Results: A significant decrease of the microbiological counts was observed during the slaughtering process in all three slaughterhouses. Considering the steps A and D, mean counts of mesophilic aerobes ranged from 4.9 to 3.1 logCFU/cm² in Sl.1 ($P < 0.05$), 3.9 to 3.6 log CFU/cm² in Sl.2 ($P > 0.05$), and 4.7 to 3.6 log CFU/cm² in Sl.3 ($P < 0.05$). It was observed significant decrease of the contamination for coliforms and *E. coli* between steps A and B ($P < 0.05$), without significant differences between the remaining steps (B, C, and D, $P > 0.05$), in all slaughterhouses. The same reduction profiles were found for Enterobacteriaceae, except in Sl.2 where no significant differences were observed between all steps ($P > 0.05$). Sl.1 presented the highest contamination levels of all researched hygiene indicators in step A when compared to Sl.2 and Sl.3, and the end carcasses presented similar levels of contamination in all slaughterhouses.

Significance: The present study demonstrated how the slaughtering steps can contribute to the microbiological contamination of bovine carcasses in three distinct slaughterhouses.

P2-05 Effect of Acetic and Lactic Acids on Survival of Hygiene Indicator Microorganisms and *Salmonella* Typhimurium in a Beef Cube System

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Introduction: Organic acids, such as acetic and lactic acids, represent alternative tools to control the microbiological contamination in animal carcasses during slaughtering. In some countries, such as the United States, the spraying of organic acids in bovine carcasses is allowed to reduce their microbiological contamination. However, European countries and Brazil do not allow the use of organic acids in bovine carcasses with this purpose.

Purpose: This study aims to demonstrate the effects of some organic acids over hygiene indicator microorganisms and *Salmonella* Typhimurium in a beef cube system.

Methods: Beef cubes of 2 cm³ were cut in sterile conditions and packed in sterile bags until 100 g units. Each set of three units was inoculated with a *Salmonella* Typhimurium ATCC 14029 in order to achieve approximate concentrations of 1, 10, and 100 CFU/10 g. As control, a set of three units was inoculated with the same volume of sterile distilled water. Then, one bag of each set was added to 12 ml of distilled sterile water, the second bag with 12 ml of acetic acid at 4%, and the last bag with 12 ml of lactic acid at 4%. All sets were incubated at 7 °C, and after 30 min, 24 h and 48 h, the systems were analyzed for *Salmonella* (ISO 6579), mesophilic aerobes (Petrifilm™ AC), and coliforms and *Escherichia coli* (Petrifilm™ EC). The obtained counts (log CFU/g) were compared by ANOVA and Tukey to verify significant differences between treatments and storage periods ($P < 0.05$).

Results: It was observed in beef cube systems control a significant increase of mesophilic aerobes between 30 min (5.8 log CFU/g) and 24 h (6.8 log CFU/g) ($P < 0.05$), and non significant after 48 h (6.5 log CFU/g, $P > 0.05$). In beef cube systems treated with acetic and lactic acids,

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mesophilic aerobes contamination kept stable after 30 min, 24 h, and 48 h (5.4, 5.4, and 5.5 log CFU/g, respectively, for acetic acid, $P > 0.05$; and 5.4, 5.3, and 5.4 log CFU/g, respectively, for lactic acid, $P > 0.05$). Coliforms and *E. coli* were not detected in all beef cube systems treated with organic acids, even after 30 min of incubation. *Salmonella* Typhimurium was detected in all beef cube systems in distinct frequencies, even in the ones treated with organic acids.

Significance: The present study demonstrated the efficacy of acetic and lactic acid to reduce the contamination by mesophilic aerobes, coliforms and *E. coli* in beef cube systems, and the necessity of additional studies to determine how these substances can eliminate properly *Salmonella* Typhimurium.

P2-06 Food and Environmental Safety of Pastured Poultry Processed On-farm and at a USDA-Inspected Facility

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Introduction: Pastured poultry producers who have limited access to federal inspection face a substantial barrier to the economic feasibility of such production operations. This is despite a favorable market environment that has experienced a sustained increase of consumer demand for locally produced poultry products. This absence of regulatory guidance along with the relative scarcity of studies on pastured poultry processing practices has failed to yield a record of the data that is necessary to validate the food and environmental safety of these practices.

Purpose: The primary objective of this study is to determine the food safety risk represented by pastured poultry products. This assessment will be based on data collected from pastured poultry processing operations performed at the site of production (on farm) and at a small USDA-inspected slaughter facility. Additionally, an assessment of the environmental impact of processing waste disposal practices from on-farm processing will be performed.

Methods: Soil, compost, processing wastewater and carcass rinse samples were collected and assayed for *Salmonella* and *Campylobacter.* During the first three months of this study, four visits have been made to a small USDA-inspected facility that processes pastured poultry and two visits have been made to an on-farm pastured poultry processing operation in the southeastern United States. *Salmonella* and *Campylobacter* loads were determined using the Most Probable Number (MPN) method and the direct plate method, respectively.

Results: At the USDA-inspected facility, 83% (n=40) of the carcass rinses were positive for *Salmonella* and 90% (n=40) were positive for *Campylobacter*. The mean value for *Campylobacter* counts was 4.19 CFU/ml of rinse (95% CI: 0.47, 7.92). The mean value for *Salmonella* was 1.03 MPN/ml of rinse (95% CI: 0.22, 1.84). On the pastured poultry farm, 90% (n=20) of the rinse samples were positive for *Campylobacter* and 90% were positive for *Salmonella*. The mean value for *Campylobacter* counts was 15.13 CFU/ml (95% CI: 7.35, 22.90) and the mean value for *Salmonella* was 2.13 MPN/ml of rinse (95% CI: 0.280, 3.98). The overall prevalence of *Salmonella* in environmental samples on this farm was 76% (n=21) and a 92% (n=12) prevalence for *Campylobacter*.

Significance: The findings from this study will support the ongoing sustainable agriculture initiative by providing preliminary data to farmers on the food safety and environmental impact of pastured poultry processing.

P2-07 Prevalence of Non-O157 EHEC in Australian Manufacturing Beef

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Introduction: Since 1994, *E. coli* O157:H7 has been considered an adulterant of raw beef. Whilst *E. coli* O157:H7 remains the most commonly identified enterohemorrhagic (EHEC) serotype in clinical cases, there is increasing attention on non-O157 EHEC serotypes. FSIS recently broadened the definition of adulterant to include some strains of the serotypes O26, O45, O103, O111, O121 and O145. The term pathogenic Shiga toxin-producing *E. coli* (pSTEC) has been applied to strains of these serotypes that also harbor *stx* and *eae* genes. Australia exports manufacturing beef to the USA and this product will therefore be subject to broadened testing during US import inspection.

Purpose: To determine the prevalence of pSTEC of serotypes O26, O45, O103, O111, O121 and O145 in Australian manufacturing beef.

Methods: Thirty-three Australian beef export abattoirs were sampled in this study. Surface slices of chilled manufacturing beef (1.5 kg) were collected and 375 g sub-samples were tested using one of four different commercial testing systems. Screening tests were performed according to manufacturers' instructions. All broths from samples that yielded a screening test positive for non-O157 STEC on any test were subjected to confirmatory testing. Confirmation was conducted in accordance, as far as possible, with procedures outlined in FSIS guidebook MLG 5B.01.

Results: Sixty-eight of 2,308 samples screened positive for the presence of non-O157 STEC. The rates of screen positives for individual test methods ranged from 0.7% to 6.9% (mean 2.9%). Non-O157 pSTEC was isolated from 1 (0.04%) sample with O26 the serotype recovered. *E. coli* of serotypes O26 and O145 harboring *eae* but not *stx* were also isolated during the confirmation process.

Significance: The prevalence of non-O157 pSTEC in Australian manufacturing beef appears to be very low. The available screening tests will cause a relatively large proportion of production to be held pending confirmatory tests. These data can be used to further understand appropriate risk management for pSTEC associated with the consumption of beef products.

P2-08 *Salmonella* and *Campylobacter* Populations of Poultry Carcasses during Slaughter

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Introduction: In 2011, the United States Department of Agriculture implemented new performance standards for *Salmonella* and *Campylobacter* on young poultry carcasses. This has increased the need for poultry slaughter operations to understand the impact of their processes on the pathogenic profile of carcasses.

Purpose: This study evaluated the incidence of *Salmonella* and *Campylobacter* on poultry carcasses during slaughter.

Methods: The processing steps in two poultry slaughter plants (A and B) were analyzed and included: scalding in potable water (PW; 47-54 °C), picking using PW or chlorine (30-40 ppm), New York wash in acidified sodium chlorite (ASC, 817-1039 ppm, 2.45-2.50 pH) or chlorine (40-50 ppm), evisceration including a chlorine wash (30-50 ppm), inside and outside bird wash in chlorine (40-50 ppm), online reprocessing using ASC (930-1045 ppm, 2.36-2.51 pH), hydrochilling in peroxyacids (PA, 10-14 ppm) or chlorine (40-50 ppm), and post-chiller antimicrobial wash (PCAW) with ASC (810-879 ppm, 2.45-2.60 pH). Before and after each step (4 days, 10 samples/day), bacteria were rinsed from carcasses (1 min, 400 ml buffered peptone water solution) and rinsates were analyzed for pathogen incidence (*Salmonella* and *Campylobacter*) and counts (*Campylobacter*). Data were analyzed using a chi-square (incidence) or Tukey's test (counts) in Minitab with a significance level of $\alpha=0.05$.

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Results: Immediately prior to scalding, carcasses had a *Salmonella* incidence rate of 100.0 and 20.0% for plants A and B, respectively. After processing, *Salmonella* incidence was reduced ($P < 0.05$) to 0.0% for both plants. Processing reduced ($P < 0.05$) *Campylobacter* incidence and counts on poultry carcasses from 42.1 to 2.5% and 2.2 ± 0.6 to 1.0 ± 0.0 log CFU, respectively, at Plant A and 95.0 to 26.3% and 3.6 ± 1.1 to 1.2 ± 0.7 log CFU, respectively, at Plant B. In general, individual processing steps effectively or significantly reduced ($P < 0.05$) *Campylobacter* on carcasses with scalding having biggest impact on counts (reductions of 0.7 and 1.3 log CFU for Plant A and B, respectively) and PCAW on the incidence (reductions of 27.5% and 26.2% for Plant A and B, respectively).

Significance: These data indicate that current processes, that implement multiple antimicrobial applications and control *Salmonella,* may need to be modified to control *Campylobacter*.

P2-09 Microbiological Quality of Australian Beef Primals and Manufacturing Meat

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Introduction: In 2011, the fourth national baseline study was undertaken in which frozen beef trim and, for the first time, beef primals were sampled from 29 export-registered establishments.

Purpose: The objectives of the study were to set a contemporary baseline for microbiology of beef primals and frozen manufacturing beef.

Methods: Beef primals (striploins and outsides) were sampled by sponging just prior to packaging, and frozen manufacturing meat by drilling core samples. Samples were transported chilled to a laboratory accredited to ISO-17025 and indicator organisms and pathogens tested using internationally accepted methods.

Results: Frozen boneless beef cartons (n=1,165) were found to have a mean aerobic plate count (APC) of 2.2 log CFU/g and the mean count for the 2.1 % of samples with detectable *E. coli* was 1.3 log CFU/g. The mean APC for striploins (n=572) and outsides (n=572) were 1.25 and 1.51 log CFU/cm^2, respectively. *E. coli* was isolated from 10.7% and 25.5% of striploins and outsides, respectively with mean counts of -0.49 and -0.26 log CFU/cm^2 on positive samples. *E. coli* O157:H7, *Salmonella* and *Campylobacter* were not isolated from any primal cut samples and *Salmonella* was not isolated from any of the boneless product (*E. coli* O157 was not tested because boneless meat is routinely tested). *Listeria* sp. were not detected in any of the boneless product and was isolated on 1 striploin sample. Coagulase positive staphylococci were isolated from 3.4% of boneless samples, 7.7% of striploins and 8.4% of outsides with positive samples having mean counts of 1.9 log CFU/g, 0.2 log CFU/cm^2 and 0.2 log CFU/cm^2, respectively.

Significance: The prevalence of pathogens and counts of indicator organisms on Australian primals and frozen boneless beef appears to be very low, which accords with findings from earlier baseline studies.

P2-10 Comparison of Sensitivity of Shiga Toxin-producing *Escherichia coli* Serotypes Inoculated on Beef Trimmings to Various Chemical Decontamination Treatments

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Introduction: There are numerous reports on the efficacy of various chemical decontamination treatments for beef trimmings; however, in most of these studies *Escherichia coli* O157:H7 was the target pathogen. Data are, thus, needed on whether these antimicrobial interventions are also effective against other pathogens of recent concern in fresh beef, such as non-O157 Shiga toxin-producing *E. coli* (nSTEC).

Purpose: Six chemical decontamination treatments for beef trimmings were evaluated for their effects against *E. coli* O157:H7 and six nSTEC serotypes.

Methods: Trimmings (10 cm length × 5 cm width × 1 cm thickness; approximately 100 g) fabricated from fresh beef chuck rolls were separately inoculated (3-4 log CFU/cm^2) with 4-strain mixtures of rifampicin-resistant nSTEC serotypes O26, O45, O103, O111, O121 and O145, or rifampicin-resistant *E. coli* O157:H7. Inoculated trimmings were immersed for 30 s (or 5 s for SYNTRx 3300) in solutions (150 ml) of acidified sodium chlorite (0.1%, pH 2.5), peroxyacetic acid (0.02%, pH 3.8), sodium metasilicate (4%, pH 12.5), Bromitize® Plus (225 ppm active bromine, pH 6.6), SYNTRx 3300 (pH 1.0), or AFTEC 3000 (pH 1.2). Counts of the nSTEC serotypes, on untreated and treated samples, were statistically compared with counts of *E. coli* O157:H7. The antimicrobials were evaluated (two repetitions per antimicrobial with three samples per repetition) independently; therefore, no comparisons were made between the chemical treatments.

Results: All decontamination treatments evaluated against *E. coli* O157:H7 were generally equally ($P \geq 0.05$) effective against all six tested nSTEC serotypes. Irrespective of pathogen inoculum, treatment of beef trimmings with acidified sodium chlorite, peroxyacetic acid or sodium metasilicate reduced ($P < 0.05$) initial counts (3.1-3.9 log CFU/cm^2) by 0.7-1.0, 0.6-1.0 and 1.3-1.5 log CFU/cm^2, respectively. The remaining three antimicrobials reduced pathogen counts by 0.1-0.4 log CFU/cm^2, depending on treatment.

Significance: The findings indicated that chemical interventions used against *E. coli* O157:H7 on beef trimmings should be at least equally effective against nSTEC.

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P2-11 Effects of Antimicrobial Treatments, Surface Browning Method and Product Dimensions on *Salmonella* Contamination in Not-Ready-to-Eat, Surface-browned, Frozen, Breaded Chicken Products

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Introduction: Not-ready-to-eat (NRTE), surface-browned chicken nuggets, strips and stuffed entrees have been associated with salmonellosis outbreaks due to inadequate or no cooking of the products before consumption.

Purpose: This study evaluated the effects of antimicrobial treatments, surface browning method and product dimensions on inoculated *Salmonella* in a NRTE, surface-browned, frozen, breaded chicken product.

Methods: Fresh chicken breast meat portions (5×5×5 cm) were inoculated (5 log CFU/g) with *Salmonella* (7-strain mixture) and mixed with solutions of caprylic acid (CAA; 0.0625%)+carvacrol (CAR; 0.075%), CAA (0.25%)+ε-polylysine (POL; 0.5%), CAR (0.15%)+POL (0.5%), CAA (0.0625%)+CAR (0.075%)+POL (0.5%), or distilled water (control). Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to all treatments (5% total moisture enhancement), and then ground and formed into 9×5×3 cm, 150 g portions or 9×2.5×2 cm, 50 g portions. Samples were coated with breadcrumbs, surface-browned in an oven (208 °C, 15 min) or by deep-frying in vegetable oil (190 °C, 15 s), packaged, and stored (-20 °C, 8 days). Pathogen counts of products (two repetitions, three samples each) were statistically analyzed and independent variables included antimicrobial treatment, browning method, product dimensions, and their interactions.

Results: Total reductions of inoculated (4.9 log CFU/g) *Salmonella* in control oven-browned products were 0.7 and 3.8 log CFU/g for 9×5×3 cm and 9×2.5×2 cm samples, respectively, while reductions in control fryer-browned samples were 0.6 log CFU/g, irrespective of product size.

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Compared to the untreated control, all tested antimicrobial treatments reduced ($P < 0.05$) *Salmonella* counts, and the most effective treatment was CAR (0.15%)+POL (0.5%). Irrespective of antimicrobial treatment, pathogen reductions in fryer-browned samples were not ($P \geq 0.05$) affected by product dimensions, whereas oven browning of 9×2.5×2 cm samples resulted in higher ($P < 0.05$) reductions of *Salmonella* (reductions of 3.8 to >4.6 log CFU/g) than oven browning of 9×5×3 cm samples (reductions of 0.7 to 2.5 log CFU/g).

Significance: These findings should be useful in the selection of suitable antimicrobials, browning method and product sizes to reduce levels of *Salmonella* contamination in NRTE, surface-browned, frozen, breaded chicken products.

P2-12 *Withdrawn*

P2-13 Effect of Potassium Lactate on *Clostridium perfringens* Growth during Extended Cooling of Uncured Turkey Breasts

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Developing Scientist Competitor

Introduction: The Food Safety and Inspection Service (FSIS) of USDA has issued cooling guidelines under a directive known as Appendix B which specify chilling time and temperature limits for cured and uncured cooked meat products. Sodium lactate and potassium lactate have long been known to inhibit *Clostridium botulinum* but limited studies on *C. perfringens* inhibition have been reported.

Purpose: To determine the inhibition of *C. perfringens* during extended cooling of uncured turkey breast supplemented with potassium lactate.

Methods: Three treatments of ground, skinless, boneless turkey breast were prepared with 0 (Control), 1 or 2% potassium lactate (KL, 60% syrup, w/w), and inoculated with a three-strain mixture of *C. perfringens* spores to yield 3 log CFU/g. Individual 100-g portions were vacuum-packaged, cooked to 71.1 °C (160 °F) and transferred to programmable incubators to simulate linear cooling to 4 °C (40 °F) during a 10- or 12-hour cooling protocol. Triplicate samples were assay for populations of *C. perfringens* by plating serial dilutions on tryptose-sulfite-cycloserine at 0-time, end of cooling and at three intermediate intervals for each cooling profile. Each study was replicated three times.

Results: In Control samples without antimicrobial, average populations of *C. perfringens* increased 3.5 and 4.7 log at the end of the 10- and 12-hour chilling protocol, respectively. In contrast, the addition of 1% KL reduced growth to only a 2.0 and 2.8 log increase during the two chilling protocols, respectively, whereas 2% KL inhibited growth to less than a 0.6 log CFU/g increase under both cooling regimes.

Significance: This study confirmed that the addition of 2% potassium lactate will inhibit growth of *C. perfringens* and can be used as an alternative to sodium nitrite during extended cooling in uncured meats.

P2-14 *Withdrawn*

P2-15 Characterization of *Salmonella* Isolated from the Lymph Nodes and Feces of Cattle Presented for Harvest at a Slaughter Facility in Mexico

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Developing Scientist Competitor

Introduction: *Salmonella* survive within host immune cells, disseminate throughout lymphatic tissue and reside within lymph nodes (LNs). Because many LNs are encased within adipose tissue incorporated into ground beef, there is a need to identify how *Salmonella* colonizes the LNs in order to address this food safety threat.

Purpose: The purpose of this study was to 1) determine the prevalence of *Salmonella* isolated from LNs and feces of cattle at harvest in a Mexican slaughter facility and to 2) characterize the isolates to identify how *Salmonella* might be entering the animal and colonizes LNs throughout the body.

Methods: From each carcass (n=68), LNs (subiliac, mandibular, mediastinal and mesenteric) and feces were collected during harvest in a Mexican slaughter facility. All samples were enriched in tryptic soy broth (TSB), subjected to immunomagnetic separation (IMS), streaked on xylose lysine desoxycholate (XLD) agar and confirmed as *Salmonella* via latex agglutination. Pulsed field gel electrophoresis (PFGE) typing was performed to characterize *Salmonella* isolates from select carcasses that harbored *Salmonella* in multiple LNs and resultant patterns were analyzed to probe similarities among samples from the same animal, as well as the same tissues from different animals.

Results: The prevalence of *Salmonella* was 55.9% (95% CI: 43.8-68.0%), 91.2% (95% CI: 84.3-98.1%), 7.4% (95% CI: 1.0-13.7%), 76.5% (95% CI: 66.1-86.8%), 94.1% (95% CI: 88.4%-99.9%) for mandibular LN, mesenteric LN, mediastinal LN, subiliac LN and feces, respectively. PFGE patterns were identical for the subiliac and mesenteric LNs within the same animal, the mesenteric LN and feces within the same animal, and the mediastinal LNs among different animals.

Significance: Results suggest that *Salmonella* is commonly harbored in cattle LNs and PFGE patterns indicate that certain *Salmonella* strains may be more likely to colonize different LNs, suggesting various methods of entry. These findings should be further investigated to gain a better understanding of routes of infection and opportunities for *Salmonella* control in ground beef products.

P2-16 Comparison of Peroxyacetic Acid Treatment and Standard Hot Water Treatment for the Inactivation of Non-O157 STECs on Meat Cutting Tools

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Introduction: The practice of hot water sanitization of meat cutting tools is an internationally recognized standard. A temperature of no less than 82 °C is used to prevent cross contamination between carcasses in meat processing facilities although equivalent alternative procedures are also available. The three main disadvantages of hot water treatment are: scalds and burns to workers, entrapment of pathogenic bacteria on tools due to heat agglutination of meat proteins, and high energy costs associated with heating and maintaining water temperature.

Purpose: The purpose of this study was to determine the effectiveness of an alternative knife sanitization method using peroxyacetic acid (POA) at two temperatures.

Methods: Sterile knives were inoculated with a cocktail of five strains of nalidixic acid resistant non-O175 STEC suspended in a raw meat soil. The knives were air-dried and individually treated with a standard hot water treatment (82 °C), 220 ppm of POA at room temperature and POA at 40 °C for 1, 5, 10 and 15 s. Surviving STECs were enumerated on tryptic soy agar supplemented with nalidixic acid (TSA-NA).

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Results: Hot water treatments at 82 °C for 1 and 5 s reduced non-O157:H7 STECs by 3.64 and 5.47 log CFU/knife, respectively. POA treatment at room temperature reduced these bacteria by 4.30 log CFU/knife and by 4.74 log CFU/knife. When temperature of the POA was elevated to 40 °C reductions of 5.08 and 5.47 log CFU/knife were observed. Reductions of < 0.5 log CFU/knife were observed for water at room temperature and water at 40 °C.

Significance: The results of this study indicate that a peroxyacetic acid-based knife treatment (at 40 °C) is an alternative equivalent method to the standard hot water treatment and can reduce the cross contamination of non-O157 STECs with added advantages of lower energy costs and enhanced worker safety.

P2-17 Characterization of Antimicrobial Resistance and Virulence Genes in *Enterococcus faecalis* Isolated from a Pork Processing Plant

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Introduction: *Enterococcus faecalis* is an important pathogen often implicated in nosocomial infections and poses a major public health risk. However, little is known about phenotypic antimicrobial resistance (AMR) and AMR/virulence gene composition in *E. faecalis* isolated from commercial pork plants.

Purpose: To characterize AMR phenotypes in *E. faecalis* isolated from commercial pork processing plant, to analyze AMR and virulence genes and to describe statistical associations between AMR phenotypes, resistance and virulence genotypes.

Methods: A total of 200 samples were randomly obtained from carcasses after bleeding (BC; 50), pasteurization (PC; 100) and from retail pork products (RP; 50). One isolate from each positive sample was analysed for antimicrobial susceptibility and characterized using an enterococcal DNA microarray for analysis of resistance and virulence genes.

Results: Resistance to clinically important drugs, ciprofloxacin (one isolate each from BC and RP samples) and daptomycin (one isolate each from PC and RP samples) was found. Multiresistance (≥5 antimicrobials) was more common in *E. faecalis*isolated from BC (77.4% of isolates) samples than those from PC (25%) and RP (37.6%) samples. The most common resistance genes (> 5% prevalence) found in *E. faecalis* were aminoglycosides (*aac(6), aphA3, aadE,*), macrolides-lincosamide (*ermB, ermA, sat(4), linB*) and tetracyclines (*tetL, tetM, tetO*). The virulence genes expressing adhesion (*ace, efaAfs, agrBfs*), gelatinase (*gelE*) and pheromone (*cAM, ccF10, cob, cpd1*) factors were frequently found and isolates carrying these genes were more likely to be statistically associated with genes conferring resistance to aminoglycoside, tetracycline, erythromycin and lincomycin.

Significance: These data underscore the importance of *E. faecalis* isolates from pork plants as a reservoir of resistance and virulence genes that may pose food safety and public health risks. Statistical associations found between virulence and resistance genes suggest their possible linkage on a common genetic element, raising concerns about their potential for co-transfer to other enterococci.

P2-18 Growth of *Clostridium perfringens* from Spores in Beef, Pork and Poultry Barbeque Products

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Introduction: *Clostridium perfringens* is a pathogen of significant concern to the retail food service industry. Inadequate cooling practices and/or improper storage of meat and poultry products have been cited as a cause of numerous outbreaks of foodborne illness.

Purpose: The ability of *C. perfringens* to germinate and grow in ten commercially prepared beef, pork and poultry barbeque products was assessed.

Methods: Each product was inoculated with a cocktail of three strains of heat-activated *C. perfringens* spores to achieve ca. 2 log (low) or 4 log (high) inoculum levels, vacuum packaged, and cooled exponentially from 54.4 to 7.2 °C in 6, 9, 12, 15, 18 or 21 h, to simulate abusive cooling from the prescribed cooling time of 6.5 h. Total germinated *C. perfringens* population was determined after plating on tryptose sulfite cycloserine agar and incubating the plates anaerobically at 37 °C for 48 h. Also, *C. perfringens* growth from spores was assessed at an isothermal temperature of 44 °C.

Results: The pH of the products ranged from 4.74 to 6.35. No growth was observed in products with pH ranging from 4.74 to 5.17, both during exponential abusive cooling periods of up to 21 h and during storage for 21 h at 44 °C. While less than 1 log growth of *C. perfringens* from spores was observed in the pH 5.63 product cooled exponentially from 54.4 to 7.2 °C in 15 h or less, product with pH 6.35 supported growth even during 6 h cooling.

Significance: These challenge tests demonstrate that pH adjustment of the barbeque products to pH ≤5.63 inhibit *C. perfringens* spore germination and outgrowth during extended cooling periods from 54.4 to 7.2 °C in up to 15 h, while safe cooling for products with homogeneous, lower pH can be substantially longer.

P2-19 Characterization of Extraintestinal Pathogenic *Escherichia coli* Isolated from Retail Poultry Meats Purchased in Alberta, Canada

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Introduction: Extraintestinal *E. coli* (ExPEC) are epidemiologically and phylogenetically distinct from intestinal pathogenic *E. coli* strains and can pose serious risks to human health. The ExPEC causes between 6-8 million cases of infections costing about US$ 2 billion annually. Little is known about the prevalence of ExPEC and their complement of virulence genes isolated from retail poultry.

Purpose: The objective of this study was to investigate the prevalence of ExPEC-related genes in *E. coli* isolated from retail poultry meats purchased in Alberta.

Methods: Multiplex PCRs were used to detect 51 virulence genes in about 700 *E. coli* isolated from retail poultry meats. The ExPEC pathotype defined by the detection of two or more of the following virulence genes: *papA* and *papC, sfa, kpsMT II,* or*iutA* were then compared to 12 *E. coli* isolates recovered from clinical cases of human infection (stool, blood and urinary tract infections). Phylogenetic grouping and genetic relationships between isolates were determined by PCR and pulse field gel electrophoresis (PFGE).

Results: Fifty nine (8.4%) of the 700 *E. coli* isolates from poultry meat were identified as ExPEC, and were equally distributed among the phylogenetic groups A, B1, B2 and D. Human ExPEC isolates were from phylogenetic group A, B2 and D. Poultry isolates of phylogenetic group A possessed up to 12 virulence genes compared to 24 and 18 genes in phylogenetic groups B2 and D, respectively. Poultry meat *E. coli* identified as ExPEC harbored as many virulence genes as those from human isolates. In addition to the *iutA* gene which was found in 98% of chicken isolates, siderophore-related *ironEC* (61% of chicken isolates) and *fyuA* (yersinabactin: 100% human isolates) were detected in combination with other

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virulence genes including those encoding adhesin, protectin and toxin genes. The *papG allele I'*, *papG allele 1*, and *clpG* were not found in any of the *E. coli* isolates, while *fimH*, *ompT*, *traT*, *uidA*, and *vat,* were commonly detected. More than 66% of human ExPEC carried *papG allele II, papG allele 2&3,* and *iha* genes and about 15% poultry ExPEC harboured these genes. The *hemF*, *iss,* and *cvaC* genes were not found in human ExPECs but about 40% of poultry ExPECs carried these genes. All human ExPEC harboured *concnf* and *hlyD* which were not found in poultry ExPEC. The PFGE showed poultry ExPECs clustered with human ExPEC suggesting a genetic relationship.

Significance: Comparing ExPECs isolated from retail poultry meats with those from humans provided insights into their virulence potential that may pose serious meat safety risks. Further investigations on the ability of our poultry ExPEC to cause diseases are warranted.

P2-20 Poultry Processing Steps That Lower the Number of *Escherichia coli* on Whole Chickens May Result in a Similar Decline in *Campylobacter* Numbers

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Introduction: *Campylobacter* is a major cause of foodborne bacterial gastroenteritis in much of the western world. Poultry is considered a major reservoir of this organism. A decrease in the numbers of *Campylobacter* on chickens by log 2 at the end of processing can reduce the incidence of campylobacteriosis in humans by up to 30 times.

Purpose: The purpose of this study was to evaluate the potential of using *E. coli* counts as an indicator of changes in the numbers of *Campylobacter* through the poultry processing chain.

Methods: Whole chickens (n=10) were collected from each of five sites along the processing chain from 4 different broiler flocks at 2 abattoirs. Collection sites included: immediately before scald but after bleed-out; immediately after scald but before defeathering; after evisceration immediately before immersion chilling; after immersion chilling; and after packaging. Individual ceca (n=10) were also collected from each flock at the point of evisceration, for a total of 60 samples per flock. Whole chickens were sampled using the whole bird rinse technique following Australian Standard AS5013.20. Rinsates were enumerated for *Campylobacter* on modified charcoal cefoperazone deoxycholate agar containing antibiotics and for *E. coli*using *E. coli*/Coliform Petrifilm™. Correlation between *Campylobacter* and *E. coli* counts was assessed using Spearman's rank order coefficient.

Results: A strong association was noted between *E. coli* and *Campylobacter* counts when all sampling sites within each flock were examined, with r^2 values of 0.88, 0.89, 0.75 and 0.90 for Flocks 1 to 4, respectively. All flocks had a significant decrease in numbers of both *Campylobacter* and *E. coli* after scald and after immersion chilling.

Significance: These data suggest that the use of *E. coli* counts as an indicator of *Campylobacter* counts may represent a more applicable and practical solution to monitoring process effects, based on the relative ease and standardization of*E. coli* enumeration.

P2-21 Microbiological Profile of the Most Important Steps during the Poultry Slaughter

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Introduction: During the poultry slaughter process there are several steps where microbial levels on carcasses can change. Therefore, to know the points where contamination increases or decreases is strategically important to establish measures of control to reduce microbiological risks.

Purpose: Identify the contamination by *Salmonella*, *Campylobacter* and indicator microorganisms in main steps along the poultry slaughter in a industry located in south of Brazil to map the critical points of microbiological contamination.

Methods: Ten lots were evaluated in different days. Each day carcasses were collected before and after scalding, after plucking, before and after evisceration, from critical control point of fecal contamination, before and after chiller, scalding and chiller water (n = 130). Detection of *Salmonella* and *Campylobacter* were conducted using PCR BAX System and aerobic mesophilic (AM), *E. coli* (EC), total coliforms (TC) and *Enterobacteriaceae* (EB) using 3M Petrifilm.

Results: *Campylobacter* was not isolated in any sample. *Salmonella* was present in nine of the thirteen steps evaluated with 10% of positivity. In four of ten sampling days any *Salmonella* was detected. Detection of *Salmonella* was heterogeneous, indicating that the presence of bacteria is dependent of several factors. For indicator microorganisms statistical difference ($P > 0.05$, Tukey test) was observed between same steps for all the microorganisms tested. From beginning to the end of the process, reduction of 2.77 for AM, 3.42 for EC, 2.66 for TC and 3.36 log CFU/g for EB was observed.

Significance: The study was used to evaluate the microbiological performance of a poultry slaughter industry in Brazil and establish strategies to decrease microbiological contamination.

P2-22 Biofilm vs. Planktonic Cells: A Comparative Study on Cross-contamination Levels of Beef Filets by the Pathogenic Bacteria *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* ser. Typhimurium

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Introduction: Cross-contamination contributes to foodborne illnesses due to the potential transfer of pathogens to food products.

Purpose: To evaluate the transfer of bacterial biofilm cells in comparison with that of planktonic cells of *E. coli* O157:H7, *Salmonella enterica* ser. Typhimurium and *Listeria monocytogenes* to beef filets.

Methods: In the context of ProSafeBeef, an EU-funded project six sequential, non-inoculated beef filets came in contact with stainless steel surfaces where each of *E. coli* O157:H7 (3 strains), *Salmonella enterica* (3 strains) and *L. monocytogenes* (6 strains) had previously formed biofilm. In parallel, six sequential, non-inoculated beef filets came in contact with stainless steel surfaces contaminated by planktonic cells of each pathogen at the same levels as with biofilm cells. The time of contact between filets and surfaces was 1 or 15 min, respectively, and each experiment was replicated twice (2 batches) with three samples analyzed each time.

Results: All non-inoculated beef filets were contaminated through their contact with the stainless steel surfaces with biofilm or planktonic cells for all pathogens, regardless of contact time. The stainless steel surfaces with biofilm of*Salmonella*, for 1 minute time of contact, contaminated all samples with the sixth sample to be at the level of 4.12 log CFU/cm^2 while the corresponding population with planktonic cells was 2.95 log CFU/cm^2. In this respect, the population of *E. coli*O157:H7 in the case of biofilm and planktonic cells for the sixth sample was 2.34 log CFU/cm^2 and 0.45 log CFU/cm^2, respectively. For 15 min time of contact, *L.monocytogenes* biofilm cells contaminated the beef in higher numbers for all samples in comparison with planktonic cells.

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Significance: The results can be used to fill knowledge gaps in risk assessment studies since they provide significant insights for the risk estimation related to cross-contamination aiming thus to food safety enhancement.

P2-23 Source Tracking of *Salmonella enterica* in Broiler Production

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Introduction: *Salmonella* is one of the major causes of foodborne disease throughout the world. Among foods of animal origin, poultry is among the most common sources of *Salmonella*. To effectively control the contamination of *Salmonella*in poultry, sources of the contamination should be identified throughout the production process. Possible source of contamination can be identified by assessing clonal relationship between the suspected sources and the product of interest. Pulsed-Field Gel Electrophoresis (PFGE) was used to assess the genetic clonality of *Salmonella* isolates in this study.

Purpose: To determine the main sources of *Salmonella* introduction to broiler during production in farm level by PFGE.

Methods: Samples were collected chronologically from broiler and broiler farm environment throughout production period (6-weeks) from the same house, two times (2 flocks). *Salmonella* were isolated according to ISO 6579 method and serotyped according to the Kauffmann-White scheme. The serotypes common to both broiler and environment were selected and their clonal relationships were identified by PFGE according to the PulseNet standard protocol.

Results: In the first flock, we found three *Salmonella* serotypes common to both broiler and farm environment, i.e., *S.* Derby, *S.* Albany, and *S.* Weltevreden. The sources of these serotypes were broiler house, feed, water, and house lizards. Indistinguishable PFGE patterns were obtained from all *S.* Derby isolates from feed, water and cloacal swab; *S.* Albany isolates from farm equipments, water, and broiler samples; and *S.* Weltevreden isolates from house lizards and broiler samples. In the second flock, we found one serotype, *S.* Albany, that was common to the catching boxes before used and live broiler at slaughterhouse. However, the PFGE analysis revealed that those isolates were unrelated. Interestingly, we also found *Salmonella* isolates of the same serotype with indistinguishable PFGE patterns in house lizards from different flocks that were collected at different time, indicating that this pest may play a significant role as a continuous reservoir for the*Salmonella* in the broiler farm.

Significance: The study emphasized importance of effective cleaning and disinfecting farm environment, feed quality control, and pest management, especially house lizard, in controlling *Salmonella* in broiler.

P2-24 Analysis of Data from FSIS Sampling Programs for *Salmonella* in Cattle, Swine, and Poultry Products

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Introduction: The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) collects samples from cattle, swine, and poultry (chicken and turkey) slaughter and/or grinding establishments as part of the process verification programs. These samples are analyzed for the presence of *Salmonella* and all positive samples are further subtyped/serotyped.

Purpose: To analyze results from FSIS sampling programs in order to identify possible trends in the data, including changes in the percentage of positive samples and serotype information over time.

Methods: Samples were collected from FSIS-regulated slaughter and/or grinding establishments and were analyzed by using the FSIS Microbiology Laboratory Guidebook methods described in Chapter 4.05 and 4C.03 for screening, identification, and isolation of *Salmonella*.

Results: Data sets from March 1998 to November 2011 were used to identify trends in percentage of samples positive for *Salmonella* and to compare the most common serotypes found in the different product categories (cattle, swine, chicken, or turkey). Generally, all products showed a reduction in percentage of positive samples over this 13-year period. A comparison of the percentage of positive carcass and ground product samples identified higher levels of *Salmonella*detected in ground product. Additionally, serotype differences between species slaughtered and product types (carcass sponge/rinse versus ground meat sample) were found.

Significance: FSIS sampling programs show reductions in the percentage of samples positive for *Salmonella* for all product categories analyzed. Notably, turkey products, specifically the percentage of positive carcass samples, had the largest decrease since 2006, when FSIS turkey carcass sampling began. Data analyses also found a higher percentage of *Salmonella*-positive ground product samples compared to carcass samples for all product categories.

P2-25 From Farm-to-Fork: *Campylobacter* Immunological Rapid Screening Tool for Farm-based, Pre-slaughter Screening of Live Chicken

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Introduction: The USA and EU are increasing the focus on consumer health and food safety, especially in relation to *Campylobacter* infection. A Dutch study concluded that the risk of contracting campylobacteriosis could be effectively reduced by identifying and eliminating high shedding flocks, pre-slaughter, from fresh poultry meat production.

Purpose: For this purpose, a new lateral flow based method suitable for use on the farm by unskilled personnel and capable of delivering results within 2 hours was evaluated.

Methods: Field studies were conducted to evaluate the suitability and performance of the Merck Millipore *Campylobacter* Immunological Rapid Screening Kit compared to the standard culture ISO 10272 method, and in one study, also to real-time PCR, using a cross-seasonal representative set of fecal samples, collected during the *Campylobacter* baseline study launched by the European Commission in 2008.

Results: From a total of 187 flocks, the Lateral Flow test identified 102 *C.jejuni* and/or *C.coli*-positive flocks out of the 114 positive flocks detected by culture, indicating a sensitivity of 89.5% for the LFD. Eleven of the 12 false-negative samples were below 7.0 log CFU *Campylobacter* spp./g of feces and could be considered as not high-shedding flocks. Real-time PCR revealed 134 *C.jejuni* and/or *C.coli*-positive flocks out of 187 (71% prevalence) and so higher than identified by culture and Lateral Flow.

Significance: These studies indicate the lateral flow kit can reliably identify high shedding *C.jejuni* and/or *C.coli* broiler flocks under field conditions, pre-slaughter, with a time-to-result of 2 hours, and could be used as a tool for a*Campylobacter* Risk Management Program.

P2-26 A Novel Extraction and LAMP Assay to Detect Pork in Processed Meat Products

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Introduction: Millions of people around the world are unable to eat pork or products that contain pork due to religious or personal beliefs. Currently, the only way to tell if there is pork in a product is to read the ingredients list, which means that people must rely on the manufacturer to completely list all ingredients.

Purpose: Evogen has developed a fast and easy method to detect pork in processed meat products. This method makes use of a nucleic acid extraction chemistry and loop-mediated isothermal amplification (LAMP). This method allows for results to be available to the user in less then one hour.

Methods: Several different processed meat products were obtained from a local supermarket. Some of the products did not contain pork according the manufacturer and other products did contain pork. A one millimeter cube was cut from each product. The cube was placed into 200 µl of the nucleic acid extraction solution and incubated for 15 minutes at 95 °C. After incubation, the DNA from the lysate was used in a LAMP reaction using the Optigene Genie II and specificity of the porcine LAMP assay was determined by running exclusivity organisms including chicken, beef, and lamb.

Results: The samples that were pork-free according to the manufacturer were found to be pork-free from our test. The products that contained pork tested positive for pork with our method. These samples had a crossing threshold on average of fourteen minutes. The assay did not detect any of the exclusivity organisms.

Significance: The evaluated extraction protocol with LAMP assay delivers a quick and simple solution for detection of pork in meat products. From sample to results, detection took less then one hour to bring peace of mind to millions of people throughout the world.

P2-27 Survival of *Salmonella enterica* in Dried Turkey Manure

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Developing Scientist Competitor

Introduction: The enteric pathogen *Salmonella enterica* has been associated with foodborne outbreaks involving leafy greens. While identified as a risk factor, there have been few studies examining the role airborne transmission could play in the contamination of leafy greens. The dust associated with the application of dried poultry manure can become airborne and spread fecal contamination within fields in areas where poultry facilities are interspersed with fresh produce farms. As a first step to assessing this potential source of contamination, it is necessary to assess the likely survival of *S. enterica* in poultry manure particles of a size capable of being airborne.

Purpose: The objective of this study was to investigate the survival capabilities of two serovars of *S. enterica* Typhimurium and Enteritidis, in turkey manure dust at different moisture levels.

Methods: Fresh turkey manure (fecal material plus bedding) was dehydrated to <5% moisture content and processed until 'dust' particle sizes of approximately 125 µm were obtained. The 'dust' was sterilely rehydrated to three moisture levels - 5%, 10% and 15%, and inoculated with a cocktail of *S.* Typhimurium and *S.* Enteritidis at target levels of 10^6 CFU/ml. *Salmonella* spp. survival at each moisture level was monitored over 60 days using viable plate count methods on BHI, XLD and VRBG agars. Survival curves were plotted and basic extrapolation done.

Results: Significant ($P < 0.05$) differences were observed in the inactivation rates of *Salmonella* spp. based on moisture levels−lowest moisture level of 5% corresponded to slowest inactivation rate. Manure dust with moisture levels 5%, 10%, and 15% had, respectively, achieved log reductions of 1.75, 2.96 and 3.06 on XLD agar by day 14, and enrichments indicated survival beyond day 60 for all moisture levels.

Significance: Data obtained from this study suggest that pathogenic *Salmonella* spp. cells can for survive for extended periods when present on manure dust particles.

P2-28 Growth of *Salmonella enterica* Serovars in Pesticide Solutions and Their Survival during Production and Processing of Field-grown Tomatoes

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Introduction: Foliar contact water contaminated with human pathogens has been implicated in produce outbreaks and previously shown to support bacterial growth when mixed with some ag-chemicals.

Purpose: To evaluate *in vitro* the growth kinetics of *Salmonella enterica* in pesticides solutions and the effect of ag-chemicals applications with contaminated water to tomatoes during field production.

Methods: The effect of temperature (10, 25 and 37 °C), organic matter in water and time of incubation (0 - 96 h) on the growth of a cocktail of *S. enterica* (sv. Newport, Poona and Michigan) was determined across 13 pesticides formulations labeled for fresh tomato, following a factorial design. Pesticides that showed enhanced, neutral and inhibitory effect on the growth of *Salmonella* were selected for application to an experimental tomato field. Mature green tomatoes, were collected 3, 7 and 12 days post inoculation and analyzed for *Salmonella* with and without postharvest washing with 50 mg/l of sodium hypochlorite at pH 7.0.

Results: Positive and significant correlation ($P < 0.05$) was found between the growth of *Salmonella* and the temperature, time of incubation, pesticide and water source. Cabrio, Admire, Sulfur and Success allowed growth of *Salmonella* while Asana, Ridomil and Intrepid reduced survival. Analysis of the strain distribution of the *Salmonella* cocktail after incubation in pesticide suspensions showed a greater recovery of sv. Newport, followed by Michigan and Poona. Recovery of the bacterium from the field grown tomatoes declined from 80 to 15% after 3 and 12 d of contaminated pesticide application, respectively, but no differences among pesticides were observed. Postharvest washing with NaOCl was not efficient in removing remaining *Salmonella* but it could minimize cross contamination.

Significance: This study provides further evidence that pesticides may support the growth of *Salmonella* if introduced with source water and may elevate risk during foliar contact applications beyond that of the water source alone.

P2-29 Efficacy of Natural Antimicrobial Interventions against *Salmonella* and *Escherichia coli* on Fresh Leafy Greens

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Introduction: One-third of all foodborne disease outbreaks in the United States in 2011 were due to microbial contamination of fresh produce. Consumer demand for additive-free and microbiologically-safe food has increased over the years. Hence, there is an increasing need to investigate the efficacy of natural decontaminating agents against foodborne pathogens.

Purpose: The objective of this study was to test the efficacy of natural, cost-effective, and efficient intervention methods to decontaminate fresh leafy greens from foodborne pathogens (*Salmonella* and *E. coli*).

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Methods: The efficacy of lemon juice, lime juice, and vinegars at 5% acetic acid (white, apple cider and red wine) was tested against ~10^7 log CFU/ml *Salmonella* and *E. coli* cocktails at 0, 30, and 60 min *in vitro*. Following this, the most effective intervention (at various concentrations) was applied on fresh lettuce inoculated with ~ 10^5 CFU/g *Salmonella* and *E. coli* cocktail for a contact time of 15 min. The microorganisms were plated on Eosin Methylene Blue media (EMB) to differentiate between *E. coli* and *Salmonella* colonies.

Results: The results of the *in vitro* studies demonstrated that white vinegar was the most effective intervention against both microorganisms causing a reduction of 10^3 CFU/ml at 30 min and counts below detection limit at 60 min. White vinegar was applied at concentrations 1.25%, 2.5%, and 5% acetic acid on lettuce inoculated with ~ 10^5 CFU/g *Salmonella* and *E. coli*. The results demonstrated that the application of 2.5% and 5% white vinegar were the most effective at reducing *E. coli* and *Salmonella* by 10^2 and 10^3 CFU/g respectively.

Significance: The application of a natural antimicrobial agent such as white vinegar (1:1 dilution) on fresh produce significantly reduces the risk of foodborne pathogens such as *Salmonella* and *E. coli*. This intervention could keep the consumer safe and free from foodborne disease.

P2-30 Colonization of *Salmonella* spp. on Surface of Apple

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Introduction: Several foodborne illness outbreaks associated with fresh produce have recently ocurred. Consumption of fresh apples has not been reported as the cause of disease. However, products like apple juice and cider have been vehicles of pathogenic microorganisms.

Purpose: The aim of this work was to evaluate the ability of *Salmonella* to adhere and to colonize on the surface of apple varieties Rayada, Red Delicious and Golden Delicious cultivated in Queretaro, Mexico.

Methods: *Salmonella* strains resistant to rifampicin were used: one strain of *S.* Typhimurium, another strain of *Salmonella* spp. isolated from composta and three strains of *Salmonella* spp. isolated from an apple orchard (soil, fecal materia and apple). Rayada Red Delicious and Golden Delicious apples were inoculated (with mixture and individual strains) by partial immersion (3 h at 22 °C) in *Salmonella* suspension (7 log CFU/ml). The apples were washed with peptone diluent. Washed apples were exposed to soil suspension (10% w/v) and stored at 22 °C for 48 h. Periodically, three units of each variety were taken off and washed. Attached cells were removed and quantified in tripticase soy agar (rifampicin 200 ppm). In apples inoculated with individual strains, attached cells were only quantified at 0 and 48 h of storage. *Salmonella* adhesion and/or biofilm formation were investigated by scanning electron microscopy.

Results: Adhesion ability of *Salmonella* on the three apple varieties was similar. Adhesion percentage observed were 0.88, 0.95 and 1.27% for Rayada, Red Delicious and Golden Delicious, respectively. Maximum population reached during*Salmonella* colonization was similar among the apple varieties (5.1-5.8 log CFU/apple). The *Salmonella* strain isolated from soil and growing on Rayada apple showed the biggest population increase after 48 h (2.2 log CFU). Scanning electron microscopy observations showed a structured biofilm.

Significance: *Salmonella* showed an ability to colonize apple surface with soil solids as nutrient. Biofilm formation could improve the pathogen survival on the friut.

P2-31 Evaluation of Factors that Influence the Cross-contamination of *Escherichia coli* between Gloves and Lettuce during Harvesting

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Developing Scientist Competitor

Introduction: Gloves have been increasingly used to hand harvest fresh produce without sufficient data to support development of appropriate management practices.

Purpose: To evaluate factors affecting the transfer of *E. coli* between gloves and lettuce.

Methods: In the laboratory, leaves on Romaine lettuce plants were inoculated with approximately 6 log CFU/leaf of generic *E. coli*. After 6 or 24 h, pieces (100 cm^2) of latex gloves (disposable smooth, or reusable; smooth or textured) were held on the inoculated lettuce surface for 5 s. In Salinas, CA, field, heads of lettuce were inoculated at 7 log CFU/head and dried for 2 h. A single inoculated head of lettuce was harvested with an uninoculated glove before harvesting 20 additional uninoculated heads using the same glove. Latex gloves (disposable smooth or reusable textured) were inoculated with *E. coli* at 4.5 log CFU/glove, dried for 1 min, and used to harvest 20 heads of lettuce. Lettuce leaves and gloves were homogenized in 0.1% peptone or Dey-Engley broth, respectively, plated on tryptic soy (TS) agar or enriched in TS broth with 50 µg/ml rifampicin and incubated for 24 h at 35°C.

Results: In the laboratory, 10 to 100 cells of *E. coli* were transferred from inoculated lettuce leaves to gloves, irrespective of drying time or glove type. In the field, after harvesting a single inoculated head of lettuce, sufficient cells were transferred to the glove such that *E. coli* was detected on 20 subsequent heads of lettuce more than 50% of the time, irrespective of glove type. When gloves were inoculated, *E. coli* was transferred to a greater proportion of lettuce heads when harvested with disposable gloves than with reusable gloves.

Significance: Contaminated gloves can transfer *E. coli* to multiple heads of lettuce during harvest; in some cases glove type may impact this transfer.

P2-32 Effect of Sanitizers and Ozone Combinations against *Salmonella enterica* Typhimurium on Green Onions

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Developing Scientist Competitor

Introduction: *Salmonella* is one of the most causative microorganisms for foodborne illnesses in United States and many other countries. Four deaths and 1028 illnesses in United States have been attributed to three microbial foodborne outbreaks associated with green onions.

Purpose: The objective of our study was to investigate the efficacy of generally regarded as safe (GRAS) antimicrobial washing treatments including hydrogen peroxide (H_2O_2), SDS, organic acids (citric acid, acetic acid and malic acid), thymol, bubbling ozone as well as their combinations for inhibiting *Salmonella enterica* Typhimurium on green onions without affecting the produce quality.

Methods: *Salmonella* were surface inoculated on green onions, and various washing treatments including acetic acid, malic acid, citric acid (1-2 mg/ml), hydrogen peroxide (300-800 ppm), SDS (1-4%), thymol (0.2-0.4 mg/ml), bubbling ozone (6.25 ppm) as well as their binary combinations were investigated to inactivate *Salmonella* on green onions. The *Salmonella* population on green onions was enumerated by plating method. The antimicrobial effects of the test treatments were compared to 200 ppm chlorine, no wash treatment and water wash. Quality of green onion

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including color, texture and overall visual appearance was also evaluated.

Results: Most of our wash treatments reduced *Salmonella enterica* Typhimurium significantly compared with "no wash" control group. Among single antimicrobial treatments, 0.4 mg/ml thymol was the most effective with 4.85-log reduction and it had similar efficiency as compared to 200 ppm chlorine (4.97-log reduction). Other treatments were less effective, 2 mg/mL acetic acid, malic acid or citric acid and 6.25 ppm bubbling ozone had approximately 2-log reduction. However, all of them showed significantly stronger *Salmonella* inactivation capacity than water washing (1.07-log reduction). Combinations such as 0.2 mg/ml Thymol+2 mg/ml acetic acid (4.32-log reduction) and 300 ppm H_2O_2+4% SDS (2.02-log reduction) also provided effective activities to mitigate *Salmonella*. Ozone-SDS or ozone-hydrogen peroxide combinations significantly increased *Salmonella* log reduction. In quality tests, none of the treatments aforementioned had significant negative effect on green onions, although increase of green color has been observed in some groups.

Significance: Our work suggests a new environmentally friendly washing solution might be developed without use of chlorine to inactivate *Salmonella* on green onions.

P2-33 The PCS, D-FENS, and D-FEND_ALL: Novel Chlorine Dioxide Technologies for Military Applications in Fresh Produce Safety

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Introduction: With increased consumption of fresh fruits and vegetables, recent outbreaks of *Listeria monocytogenes* in cantaloupe, *Escherichia coli* in sprouts, and *Salmonella spp* in peanuts demonstrate the public's growing concern relating to fresh produce safety. We present an ensemble of novel chlorine dioxide technologies developed by Army researchers as their progression from exciting laboratory discoveries to commercialization, with particular emphasis on ensuring fresh produce safety, while retaining food quality and acceptance.

Purpose: Chlorine dioxide is a broad-based biocide. The objective here is to adapt the following novel chlorine dioxide technologies for food safety.

a) "Novel Chemical Combination" – novel effector chemistry system.
b) "Portable Chemical Sterilizer" - a revolutionary, energy-independent, waterless medical sterilization device for surgical instruments or fresh produce commodities.
c) "Disinfectant-sprayer for Foods and ENvironmentally-friendly Sanitation (D-FENS)" - for hard surface sanitization.
d) "Disinfectant for Environmentally-friendly Decontamination, All Purpose (D-FEND_ALL)" – to be submitted and licensed to food industry in 2012 for immediate commercial use in microbreweries, water treatment, fresh produce safety, dairy processing plants, and possibly household appliances.

Methods: The PCS was tested to inactivate *L. monocytogenes, E. coli*, and bacterial spores from whole tomatoes, and to inactivate browning enzymes in sliced apples. D-FENS was tested on *Staphylococcus aureus* on surfaces, and D-FEND_ALL was tested on spores of *Bacillus anthracis* on Army textile materials. Survivors were recovered with standard serial dilution and plate-counting enumeration techniques.

Results: In all cases, the technologies eliminated pathogens without harming surface materials or compromising the appearance of the produce.

Significance: These chlorine dioxide technologies provide technological advantages for the military and consumer markets concerned with fresh produce safety. In addition to providing safe, fresh, wholesome products, these are "green" technologies that protect the environment from carcinogens and decrease landfill wastes, fossil fuel usage, and carbon footprint.

P2-34 Transfer Potential of *Salmonella* between Cardboard Cartons and Tomatoes

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Introduction: Packing cartons may be reused during tomato harvest and packing. Presently, there is inadequate data to quantify cross-contamination risks associated with this practice.

Purpose: The objectives of this study were to determine *Salmonella* transfer coefficients (TCs) between (i) new, used and dirty inoculated cartons and tomatoes and (ii) inoculated tomatoes and new, used and dirty cartons, under varying inoculation conditions, contact times and temperatures.

Methods: A five-strain cocktail of rifampicin-resistant *Salmonella* was spot inoculated onto 5 x 5 cm carton pieces to obtain a final concentration of ca. 6 log CFU/carton. Cartons were either touched immediately to mature green tomatoes (wet) or allowed to dry for 1 or 24 h before contact. Tomato/cartons were subjected to three different contact times (touch (0), 1, and 7 days), at 25°C or 12°C. All sampling was done with 10 replicates. Bacterial populations were enumerated on tryptic soy agar supplemented with rifampicin. The transfer direction was then reversed by inoculating the tomato and contacting the carton.

Results: *Salmonella* populations decreased following inoculation onto new, used and dirty cartons by 2-3 log units during 24 h drying regardless of storage temperature. In general, the highest transfer rates occurred with wet inoculum, regardless of carton type or temperature storage. The highest TCs was a wet, inoculated tomato stored 7 days at 25°C, TC=14.7. Increasing contact time decreased TCs for new cartons, but increased TCs for used and dirty cartons. A greater percentage of *Salmonella* transferred from tomatoes to cartons than from cartons to tomatoes, regardless of carton type or storage temperature. For example, TC=0.27 from a wet, new carton, immediately touched to a tomato; while TC=1.38 from the tomato to the carton under the same conditions.

Significance: *Salmonella* transfer between tomatoes and tomato cartons varies between new, used and dirty tomato cartons, indicating cross-contamination risks may increase under some conditions when cartons are dirty or reused.

P2-35 Inactivation of *Listeria innocua*, *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on the Surface and Stem Scar of Tomatoes Using In-package Ozonation

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Introduction: The microbial safety of fresh fruits and vegetables continues to be a major concern as produce recalls and foodborne illness outbreaks associated with their consumption occur every year. Novel intervention technologies are needed to enhance microbial safety of fresh produce.

Purpose: The objectives of this present study were to evaluate a novel in-package ozone generating system (PlasmaLabel) for its effectiveness in inactivating *Listeria innocua*, and attenuated *E. coli* O157:H7 and *Salmonella* Typhimurium on tomatoes and maintaining fruit quality.

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Methods: The three bacteria were inoculated either on the smooth surface of the tomato or the stem scar area before being sealed in plastic film bags and subjected to treatment using the PlasmaLabel system for various times (up to 4 min). Non-inoculated fruit were used to assess quality changes during 21 day post-treatment storage at 10°C.

Results: The PlasmaLabel system was able to produce ozone inside of sealed film bags, reaching a concentration of 1000 ppm within 1 min of activation. When *L. innocua* was inoculated onto the smooth surface of the fruit, the bacterium was reduced to a non-detectable level within 40 sec of treatment and was reduced by 4 log CFU/fruit on the stem scar area. Increase in treatment time did not result in a proportional increase in bacterial inactivation. For *E. coli* and *Salmonella*, there was little difference (<1 log) in the effectiveness of the system regardless of inoculation site. Both bacteria were typically reduced by 2-3 log CFU/fruit after a 2-3 min treatment. Ozonation resulted in no significant negative effects on fruit color or texture observed during the 22-day post-treatment storage at 22°C.

Significance: These results suggest that *Salmonella, Listeria,* and *E. coli* respond differently to ozonation. Ozonation may be a good alternative to aqueous sanitizers for enhancing microbial safety of tomatoes.

P2-36 Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on Bruised and Intact Strawberries

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Introduction: Strawberries are harvested at or near full ripe maturity for superior eating quality. These fruit tend to be less firm and are more susceptible to bruising during harvest and transport, which may increase risks of foodborne pathogen proliferation.

Purpose: The objective of this research was to quantify the fate of *Escherichia coli* O157:H7 and *Salmonella* on bruised and intact strawberries at shipping (2°C) and retail display (15.5°C) temperatures.

Methods: Strawberries were bruised by dropping a 32.6 g steel ball into a 23 cm PVC pipe directly onto a whole strawberry. Twenty microliters of a five-strain cocktail of rifampicin-resistant *E. coli* O157:H7 or *Salmonella*, at a concentration of ca. 10^6 CFU/ml, was spot inoculated onto either bruised or nonbruised portions of the strawberry and dried for 1 h. Strawberries, stored at 2°C and 15.5°C, were sampled at 0, 2, 5, and 24 h and days 0, 1, 3, and 7, respectively. Pathogen populations were enumerated on selective and non-selective media supplemented with rifampicin.

Results: Populations of *E. coli* O157:H7 and *Salmonella* decreased under all experimental conditions. At 2 °C, *E. coli* O157:H7 populations decreased by 1 and 0.7 log CFU/berry over 24 h on bruised and intact strawberries, respectively. *Salmonella* populations decreased by 1.3 and 1.5 log CFU/berry over 24 h on bruised and intact strawberries, respectively at 2 °C. At 15.5 °C, *E. coli* O157:H7 and *Salmonella* showed similar trends over 7 days, where both populations decreased by >2.3 and >2 log CFU/berry on bruised strawberries and >1.9 and >1.6 log CFU/berry on intact strawberries, respectively.

Significance: Bruising did not significantly affect the fate of *E. coli* O157:H7 or *Salmonella* populations on mature strawberries. The current practice of harvesting strawberries at full ripe maturity, whether bruising occurs or not, does not impose any additional food safety risks.

P2-37 Antimicrobial Activity of Oregano Oil against *Salmonella enterica* on Organic Leafy Greens at Varying Exposure Times and Storage Temperatures

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Introduction: *Salmonella enterica* has caused a number of foodborne illness outbreaks in fresh produce. Alternatives to chemical sanitizers are needed for washing organic fresh produce, since only materials approved by the USDA National Organic Program are allowable in organic production.

Purpose: The objective of this study was to evaluate the effectiveness of oregano essential oil on four different types of organic leafy greens (organic iceberg and romaine lettuces and mature and baby spinach) inoculated with *Salmonella*Newport. The difference in exposure times of treatments as well as storage temperatures was also investigated.

Methods: Each leaf sample was washed, dip inoculated with *S.* Newport (10^6 CFU/ml) and dried. Oregano oil was prepared at 0.1, 0.3 and 0.5% concentrations (v/v) in sterile phosphate buffered saline. Inoculated leaves were immersed in the treatment solution for 1 or 2 min, and individually incubated at 4 or 8 °C. Samples were taken at day 0, 1 and 3 for enumeration of survivors.

Results: Our results showed that oregano oil was effective against *S.* Newport at all concentrations. Romaine lettuce, iceberg lettuce, mature spinach and baby spinach samples showed between 0.7-4.8 log, 0.8-4.8 log, 0.8-4.9 log, and 0.5-4.7 log CFU/g reductions in *S.* Newport, respectively, when compared to the PBS control over the course of the experiment. The antibacterial activity also increased with exposure time. Leaf samples treated for 2 min generally showed greater reductions (by 1.4-3.2 log CFU/g) than those samples treated for 1 min; however, there was minimal difference in the activity among the samples stored under refrigeration and abuse temperatures.

Significance: This study demonstrates the potential of oregano oil to inactivate *S.* Newport on organic leafy greens.

P2-38 Microbial Survey of Fresh Produce Obtained from Retail Stores on the Eastern Shore of Maryland

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Introduction: The presence of pathogenic microorganisms in fresh produce, especially leafy vegetables, has raised serious concerns among the general public and governmental agencies. Recently, several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh produce.

Purpose: The objective of this study was to investigate the microbiological quality of six types of fresh produce obtained from three retail stores located on the Eastern Shore of Maryland.

Methods: A total of 414 samples representing basil, cilantro, lettuce, scallion, spinach and parsley were analyzed for total aerobic bacteria (APC), total coliforms, *Escherichia coli*, and three pathogenic bacteria (*Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*) using standard methods. Presumptive pathogenic isolates were confirmed using BAX Polymerase Chain Reaction.

Results: All samples were positive for APC, 39% positive for total coliforms and only 10% positive for *E. coli*. Mean log CFU/g for APC in the six produce types ranged from 7.49 to 8.06. The rate of detection of total coliforms and *E. coli* in the six produce types ranged from 22%-45% and 8.7%-17%, respectively. There was a statistically significant difference with respect to rate of detection of total coliforms across retail stores, but not with respect to abundance of APC or rate of detection of *E. coli*. *Escherichia coli* O157:H7 and *L. monocytogenes* were detected in one spinach sample each, while one parsley and one cilantro sample were positive for *Salmonella.*

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Significance: Although the results of this study provided some indices of sanitary and/or spoilage level, no relationship existed among the APC, total coliforms, *E. coli*, and the presence of pathogenic bacteria in the samples tested. The results of this study provide a baseline for further studies in the area to evaluate the microbiological quality of produce both sold and produced locally, as well as, to help develop risk management policies in the US.

P2-39 Temperature-dependent Survival of Hepatitis A Virus in Contaminated Green Onions

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Introduction: Pre- or post-harvest hepatitis A virus (HAV)-contaminated green onions have been linked to large numbers of foodborne illnesses. Understanding HAV survival on fresh onions after contamination would assist in projecting the risk of the disease associated with consumption.

Purpose: This study defined HAV inactivation rates in green onions stored at 3 °C, 10 °C, 14 °C, and 23 °C.

Methods: Each fresh whole onion was inoculated with 20 µl of HAV, air-dried for 20 min, packed, and stored in a designated temperature between 3 and 23 °C. At each sampling, HAV was eluted by 90 sec-pulsifying in 1% beef extract containing 100 mM Tris, and 50 mM glycine, pH 8. Infectious HAV levels in eluates were determined by performing a plaque assay on confluent FRhK-4 cells. A total of 3-7 onion samples were examined at each sampling.

Results: Increase in storage temperature resulted in greater inactivation rates of HAV on onions. A reduction of 0.034 log per day was found at 3.1 ± 1.8 °C versus that of 0.185 log per day at 23.4 ± 0.7 °C. Regression correlation coefficients between the log reduction of HAV and storage temperature ranged from 0.80 to 0.98 for 8 trials. The decimal reduction times (in day) of HAV on contaminated onions were $D_{3.4°C}$ = 30, $D_{10.3°C}$ = 14.4, $D_{14.2°C}$ = 11.4, $D_{20.2°C}$ = 6.6, and $D_{23.4°C}$ = 5.4. Overall a temperature increase by one degree Celsius would increase inactivation of HAV by 0.007 log per day (r^2=0.97).

Significance: These data may be useful in analyzing and predicting the risks associated with HAV-contamination in green onions.

P2-40 Microbial Quality of Fresh Produce: Impact of Farming Operations and Readiness for Traceability Requirement

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Introduction: Fresh produce is increasingly implicated in foodborne outbreaks, like the ones recently reported with *L. monocytogenes*, *E coli*, and *Salmonella* spp. in cantaloupe, sprouts, and peppers, respectively. Since agricultural practices for production of fresh produce have changed, the potential of microbial risk also has evolved and increased.

Purpose: The objectives of this study were to identify the risk of pathogenic *Salmonella spp (SS).* and *E. coli* O157:H7 (*ECH*) contamination on fresh produce grown using different farming practices (high tunnels and open fields) and determine the readiness of North Carolina (NC) farmers for adoption of fresh produce traceability system.

Methods: Soil and water samples were collected from 12 small-scale farms in NC and inoculated onto selective media (TSA for total aerobic count, XLT4 for *SS.,* and MacConkey for *ECH).* All plates were incubated at 48 hours at 37 °C. Following incubation, colonies were counted and the numbers were expressed as log CFU/ml. The identification of microorganisms was carried out by multiplex PCR analysis. The readiness of NC farmers for fresh produce traceability was tested using a quantitate survey on 22 farmers spread throughout the state.

Results: The results indicated that soil samples collected from high tunnel farms had the highest microbial load (8.26 log CFU/ml on TSA, 7.8 log CFU/ml on MAC, 7.3 log CFU/ml on XLT4). Both water and soil samples collected from open-field farms, especially those located in the western part of the state, had the lowest microbial counts, indicating that humidity and temperature directly affect the microbial content of soil and irrigation water. The PCR analysis confirmed the presence of *SS* only in soil samples collected from a farm located in the eastern part of the state. The traceability survey results demonstrated that 85% of farmers who participated in the survey were not familiar with the produce traceability system and traceability concept/requirement. All 22 farmers wanted to be trained on produce traceability and be able to implement the system to improve the safety of their produce.

Significance: Based on these results, improvements are needed to prevent pathogenic contamination in different farming operations to enhance produce safety. The small-scale produce farmers are interested in traceability and other ways to improve product quality and safety. Hence, they should be trained on produce traceability and best practice farming operations that increase farm profitability and sustainability.

P2-41 Behavior of *Salmonella* spp. on Fresh-cut Dragon Fruits at Different Storage Temperatures

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Introduction: Dragon fruit is one of the popular tropical fruits in Southeast Asia. Although there has been no outbreak associated with dragon fruits, the likelihood of foodborne outbreak still exists since *Salmonella* spp. have been isolated from the fruits in Malaysia. Thus, it is important to understand the behavior of *Salmonella* spp. on fresh-cut dragon fruits during storage at various temperatures in order to minimize the risk of salmonellosis.

Purpose: The aim of this study was to determine the survival or growth of unadapted, acid-adapted and cold-stressed *Salmonella* spp. on fresh-cut dragon fruits at different storage temperatures.

Methods: The dragon fruits were sliced and spot inoculated with a five-strain cocktail of *Salmonella* spp. at two inoculum levels (2.5 log CFU/g and 5.5 log CFU/g). Inoculated fruits were air dried and stored at 28 °C for 48 h and at 4 °C and 12 °C for 96 h.

Results: *Salmonella* populations significantly ($P < 0.05$) increased by 2.5 to 3 log CFU/g at low inoculum level, whereas the numbers increased by 0.3 to 0.7 log CFU/g at high inoculum level on fresh-cut dragon fruits held at 28 °C for 48 h. Only unadapted control and acid-adapted cells grew with 0.7 to 0.9 log increase at low inoculum level at 12 °C for 96 h. No significant growth was observed at both inoculum levels during the storage at 4 °C. Overall, acid or cold adaptation of*Salmonella* spp. did not show better survival or growth on fresh-cut dragon fruits during storage compared with unadapted control cells.

Significance: These results showed that *Salmonella* spp. could grow on fresh-cut dragon fruits under inappropriate storage conditions, indicating that fresh-cut dragon fruits could be a potential vehicle for salmonellosis. Thus, this study suggests that fresh-cut dragon fruits should be stored at 4 °C to inhibit the proliferation of *Salmonella* spp.

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P2-42 Impact of Pig Slurry and Irrigation on Broccoli Safety: Field Experiment

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Introduction: Irrigation water and organic fertilizers are considered as potential preharvest sources of pathogenic and indicator microorganisms on crops. However, few studies were conducted on the combined effect of both inputs.

Purpose: The aim of this study was to evaluate the prevalence of pathogenic and indicator microorganisms on broccoli fertilized with pig slurry or mineral fertilizer and irrigated 0, 1 or 2 times with contaminated water.

Methods: Twenty-four broccoli experimental plots were set using a split-plot factorial design with the fertilizer as the main plot factor and the number of irrigations as the subplot factor. Irrigation water was artificially contaminated with bovine slurry. Water samples were taken during irrigation, and broccolis were sampled 1, 3 and 5 days after irrigation. Soil samples were also taken on the last sampling day. Samples were analyzed to determine generic *E. coli* populations, and presence of generic *E. coli, Salmonella* spp., *Listeria monocytogenes*, and verotoxigenic *E. coli.* A generalized linear mixed model using SAS GLIMMIX procedure was performed to evaluate the impact of the treatments on *E. coli* prevalence.

Results: Generic *E. coli* populations in irrigation water were 393 and 538 CFU/100 ml for the first and second irrigation, respectively. *Salmonella* spp. was detected in pig and bovine slurries but no pathogenic microorganisms were recovered in water, broccoli or soil. Eleven broccoli samples were *E. coli*-positive using the enrichment procedure (n=72). None came from non-irrigated plots. *E. coli* prevalence on slurry-fertilized broccoli was 25 and 33 % when 1 and 2 irrigations were performed, respectively (n=12). *E. coli* prevalence on mineral-fertilized broccoli was 17 % for both irrigation treatments (n=12). However, statistical analysis showed no impact of treatments on *E. coli* prevalence. All soil samples taken in slurry-fertilized plots were *E. coli*-positive (n=12), while 9 out of 12 soil samples were positive in mineral-fertilized plots.

Significance: This study highlights the limited prevalence of *E. coli* on broccoli after irrigation, even if bacteria were frequently detected in soil.

P2-43 Transfer of *Enterobacter aerogenes* from Surface to Flesh during Peeling of Raw Carrots and Cucumbers

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Introduction: Fresh produce may be washed and disinfected after harvest and/or at home before consumption. It is known that microorganisms are mainly located on the surfaces of these products and that washing is not 100% effective in removing any contamination that may be present. Knowledge of bacterial transfer from the surface to the flesh during peeling is essential in understanding the role that cross-contamination may play in produce-related outbreaks.

Purpose: The purpose of this study was to determine the transfer of *E. aerogenes* from the surface of carrots and cucumbers to the flesh during the home peeling process.

Methods: A non-pathogenic nalidixic acid-resistant strain of *E. aerogenes* was grown overnight in tryptic soy broth at 37 °C, centrifuged and resuspended in sterile water. Fresh cucumbers and carrots were dipped in 200 ml of sterile water containing *E. aerogenes* for 15 min. Produce was allowed to dry for 15 min in a laminar flow hood. *E. aerogenes* was enumerated from the inoculum water, the removed peel and from the peeled produce using duplicate plates of MacConkey agar added with nalidixic acid. Counts were expressed as log CFU/g or ml and experiments were replicated.

Results: Counts of *E. aerogenes* in the water used to inoculate produce items were ~10^7CFU/ml. The mean counts of *E. aerogenes* in the removed peels of carrots and cucumber were 4.8 ± 0.2 and 3.8 ± 0.4 log CFU/g, respectively. It was observed that after peeling, internal parts of cucumber (mesocarp) and carrot (cortex) were contaminated with 2.6 ± 0.3 and 2.7 ± 0.4 log CFU/g, respectively. Contamination on the peeled flesh was likely due to transfer via the peeler blade during peeling.

Significance: Our results show that if the surfaces of cucumbers or carrots are contaminated with bacteria prior to peeling, the peeling process reduces but does not eliminate the presence of those same bacteria on the peeled flesh. These results may be helpful in understanding the risks posed by contaminated carrots or cucumbers.

P2-44 Enteric Virus Survival on Alfalfa Seeds and Sprouts

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Developing Scientist Competitor

Introduction: In addition to the numerous outbreaks of foodborne illness associated with alfalfa sprouts, the recent outbreak of *E. coli* O104:H4 in fenugreek seeds increased awareness for seed and sprout safety. Studies focused on understanding the interaction of pathogens with seeds and prevention have focused on bacterial pathogens; however, enteric viruses cause over 5 million illnesses each year in the US and are a concern for produce contamination via contaminated water or infected food handlers. Human norovirus and hepatitis A virus are the most common causes of foodborne illness by enteric viruses. Using alfalfa seeds as a model, the interaction and survival of enteric viruses was assessed.

Purpose: The objective of this study was to determine the persistence of enteric viruses on alfalfa seeds during prolonged seed storage and to determine the risk associated with contaminated seeds during germination in sprouts and germination water.

Methods: Alfalfa seeds were sterilized and inoculated with human norovirus surrogates (murine norovirus (MNV) and Tulane virus (TV)) and hepatitis A virus (HAV). Seeds were stored for 20 days at 22 °C and sampled every 5 days. Seeds were analyzed for virus presence using quantitative RT-PCR and infectivity assays. Seeds were germinated and virus presence was assessed on sprouts and in germination wastewater. Virus survival in HBSS served as a positive control and the negative control was non-inoculated alfalfa seeds.

Results: MNV titer on alfalfa seeds decreased over 20 days from 7.05 ± 0.50 log PFU/g on day 0 to 1.30 ± 1.84 log PFU/g. HAV and TV persisted on seeds over the course of 20 days with similar reductions in titer to MNV. MNV and HAV were detected in water used to germinate the seeds at 2.39 ± 0.56 log PFU/ml and 3.75 ± 0.84 log $TCID_{50}$/ml, respectively. Viruses were found to be present on sprouts after germination.

Significance: Enteric viruses persisted on alfalfa seeds prior to sprouting and pose a threat for contamination of sprouts during germination.

P2-45 Effect of Chlorine Dioxide Treatment Followed by Storage in a Modified Atmosphere on Inactivating *Cronobacter* spp. on Radish Seeds

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Introduction: Seed sprouts are often contaminated with foodborne microorganisms, since they are frequently consumed raw. *Cronobacter* spp. have been detected on various types of food including fresh produce.

Purpose: This study evaluated the inactivation of *Cronobacter* spp. on radish seeds using ClO_2 treatment followed by storage in a modified atmosphere for up to 4 days.

Methods: Radish seeds were inoculated with *Cronobacter* spp. (5 log CFU/g) adapted to nalidixic acid and treated with ClO_2 solution (0, 50, or 100 ppm). Then, the seeds were stored in a modified atmosphere (MA) (air; O_2 10%/$CO_2$10%/N_2 80%; O_2 5%/CO_2 10%/N_2 85%; or O_2 10%/CO_2 0%/N_2 90%) for up to 4 days.

Results: The *Cronobacter* spp. (4.8 log CFU/g) population on the radish seeds was reduced to 3.1, 2.8, and 2.5 log CFU/g after treatment with 0, 50, and 100 ppm ClO_2, respectively, and to 2.7, 1.8, and 0.8 log CFU/g after 2 hours of drying. After 4 days of storage under a MA, no *Cronobacter* spp. were detected on the seeds treated with 100 ppm ClO_2 solution. On the seeds treated with sterile water, the population began to decrease after 2 days of storage in a MA, but was not significantly different from that on seeds stored in air for 4 days. More than 90% of the radish seeds germinated after ClO_2 treatment, regardless of the ClO_2 concentration.

Significance: These results indicate that seeds should be treated with sanitizers at an appropriate concentration. In addition, the treatment of the seeds is more critical than the storage conditions for enhancing microbial safety.

P2-46 Effectiveness of Pulsed-Ultra Violet Light on Biofilms Formed by *Escherichia coli* O157:H7 and *Listeria monocytogenes* - A Comparative Study

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Developing Scientist Competitor

Introduction: Formation of biofilms poses significant food safety threat to food industry as a major reservoir of pathogens in food products.

Purpose: The objective of this study was to elucidate the effectiveness of PUV irradiation treatment on biofilms formed by *E. coli* O157:H7 and *Listeria monocytogenes* on food packaging material and romaine lettuce (*Lactuca sativa*).

Methods: Overnight cultures of *E. coli* O157:H7 EDL933 and *Listeria monocytogene* V7 were diluted in growth media. One milliliter of diluted culture was transferred to the petri dishes containing pieces of romaine lettuce and polyethylene packaging films, 3 x 3 cm in size, and incubated under static conditions at 30 °C for 48 h where the pieces were submerged in media containing the bacterial cultures. The formation of biofilm was confirmed by crystal violet staining. Biofilms were treated with pulsed ultra violet light using a Xenon SteriPulse 3000-XL pulsed UV light system. The samples were placed at distances of 4.5 and 8.8 cm away from the UV strobe, and treated with pulses for either 10 or 20 seconds. Reduction of the biofilm was determined by standard plating procedures following serial dilutions.

Results: The *L. monocytogens* count of biofilms formed on lettuce surface was reduced 2.1 to 3 log for 10 and 20 second treatments, respectively. While *E. coli* O157:H7 count was reduced by 2.7 to 4.5 log for 10 and 20 second treatments, respectively. Similar results were observed for biofilm reduction on the polyethylene packaging film surfaces. Our results indicate that PUV has a moderate effect in reducing *E. coli* O157:H7 and *Listeria monocytogenes'* population in biofilms on contact surfaces and fresh produce. It was also found that PUV treatment was more effective in reducing *E. coli* O157:H7 biofilms as compared to *Listeria* biofilms.

Significance: PUV light treatment has moderate effect on reducing biofilms formed by *E. coli* O157:H7 and *Listeria monocytogenes* on food packaging material and romaine lettuce surfaces. The results of the present study indicate that PUV can be a non-thermal intervention method of choice that can be used for surface decontamination of produce and packaging materials.

P2-47 Minimizing *Salmonella* Contamination in Sprouts by Controlling the Germination Temperature

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Developing Scientist Competitor

Introduction: Since 1996, contaminated sprouts have been linked to at least 30 outbreaks and over 2,000 cases of illness in the U.S. Seed is often the source of contamination, but sprouts pose a particular concern as the conditions that promote germination of the seed also promote the growth of pathogens. FDA has recommended that seeds destined for sprout production be disinfected with one or more treatments such as 20,000 ppm of calcium hypochlorite, $Ca(OCl)_2$. But this treatment is unable to completely eliminate pathogens on seeds and surviving ones can grow to significant numbers during sprouting. Thus, controlling the germination conditions such that proliferation of pathogens, if present, can be prevented or minimized is a crucial step in the overall approach to reduce microbial food safety hazards in sprouts.

Purpose: This study examined how the proliferation of *Salmonella* is affected by the temperature used for sprouting, and how this temperature effect is influenced by the pathogen load, seed lot used, and seed disinfection with 20,000 ppm$Ca(OCl)_2$.

Methods: Alfalfa seeds artificially inoculated with ~3 log CFU/g of *Salmonella* were used to prepare the contaminated seeds for sprouting. 200 g of seeds spiked with different levels (0.1, 1, or 10 % by weight) of inoculated seeds were allowed to germinate in glass jars for 3 days at 10, 20, and 30 °C. The same growth studies were performed on seeds treated with 20,000 ppm $Ca(OCl)_2$ for 15 min. Sprout samples were taken from each jar daily and analyzed for the level of*Salmonella*, either by plating on XLD plates or by the three-tube most probable number method as described in the FDA BAM.

Results: The level of *Salmonella* increased during sprouting at all three temperatures and reached the highest level at 48 h. Sprouting at a lower temperature resulted in a smaller increase in *Salmonella* counts. At a 1% spiking level, the level of *Salmonella* increased by approximately 1.5, 4, or 6 log CFU/g in sprouts grown at 10, 20, or 30 °C, respectively. Similar increases were observed during germination of seeds inoculated at a 0.1% level. Using a seedlot with a higher background microflora (~ 2 log higher), the level of *Salmonella* reached at 48 h in sprouts grown at 20 and 30 °C was slightly lower than those observed in sprouts grown from seeds with a lower level of background microflora. Treatment with $Ca(OCl)_2$ caused an initial reduction in *Salmonella* counts on seeds and a delay in the proliferation of *Salmonella* where the highest level was observed at 72 h of sprouting.

Significance: Re-growth of *Salmonella* occurred during germination of seeds that have been treated with 20,000 ppm of $Ca(OCl)_2$. Lowering the sprouting temperature could reduce the extent of pathogen proliferation.

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P2-48 The Role of Cellulose in Attachment of Shiga Toxin-producing *Escherichia coli* to Lettuce and Spinach in Different Water Hardness Environments

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Developing Scientist Competitor

Introduction: Cellulose is a linear polysaccharide polymer composed of D-glucose with β(1→4) glycosidic bond and usually exists in bacterial exopolysaccharides (EPS). Shiga toxin-producing *Escherichia coli* (STEC) plays an important role in bacterial foodborne pathogens. However, the role of cellulose on attachment of leafy greens is still unclear.

Purpose: This study was undertaken to investigate the attachment of cellulose-deficient derivates of STEC to lettuce and spinach in different water hardness environments.

Methods: Two cellulose-producing wild-type STEC strains 19 and 49 as well as their cellulose-deficient derivatives were used. Strain 49 also produces EPS comprised of colonic acid. Viability of cells was determined by plate counts on the surface and cut edge after leaves were inoculated with 10^8 CFU/ml cells at 4 °C for 2 hours. Hydrophobicity and surface charge were also determined.

Results: Strain 49 attached 0.3 and 0.6 log greater to surface and 0.9 and 0.4 log greater cut edge of spinach compared to strain 19 in wild-type and cellulose-deficient cells. In addition, there was significantly greater attachment for cellulose-proficient cells on lettuce surface than cellulose-deficient cells, but not on spinach surface. Contrary to surface results, more cellulose-deficient cells attached (0.66 and 0.3 log greater) in strain 19 and 49 to cut edge of lettuce than cellulose-proficient cells. Strain 19 was more hydrophobic than strain 49. In addition, there is progressive ordering with decreasing attachment of strain 49 in water hardness from 0 to 150 ppm, and afterwards from 200 to 1,000 ppm in order progressively increasing attachment of cells on lettuce and spinach leaves surface. The zeta potential of strain 49 was more negative than strain 19.

Significance: Cells with EPS-containing colonic acid and less hydrophobic have more potential to attach surface and cut edge of spinach as compared to cellulose-producing cells with more hydrophobic. In high water hardness environment, attachment of cells to leafy green surfaces can also be enhanced.

P2-49 The Fate of *Salmonella* Typhimurium in Foliar Fertilization Solutions in Groundwater under Different Storage Temperatures

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Developing Scientist Competitor

Introduction: During produce production, pathogen contamination may occur when water is used to apply foliar fertilizers on edible portions of fruits and vegetables.

Purpose: This study evaluates the fate of *Salmonella* in foliar fertilizers at different storage temperatures.

Methods: Rifampicin-resistant *Salmonella* Typhimurium LT-2 was inoculated at 4 log CFU/ml in fertilizers diluted with groundwater to the lowest recommended spray concentrations in Florida. Fertilizers included calcium nitrate (5 lb/acre), copper sulfate (2 lb/acre), ferrous sulfate (2 lb/acre), manganous sulfate (2 lb/acre), sodium molybdate (0.25 lb/acre), Solubor (1 lb/acre), zinc sulfate (2 lb/acre), and a groundwater control. Fertilizers were stored under refrigeration (4 °C) and temperatures common in a normal Florida growing season (15 °C or 30 °C) for 7 days. *Salmonella* populations were enumerated on rifampicin supplemented tryptic soy and bismuth sulfite agars.

Results: In all the fertilizers at all temperatures, gradual population reductions were equal to or greater than that the control. At all temperatures, *Salmonella* populations fell below the limit of detection in copper sulfate, ferrous sulfate, and calcium nitrate within 24 h, and in zinc sulfate within 72 h. *Salmonella* populations decreased by 1.4 logs in groundwater during the 7 day storage at 4 °C. The largest decline (3.2 log CFU/ml), where *Salmonella* populations were still detectable, was in molybdate. Over 7 days at 15 °C, *Salmonella* populations in ground water decreased 1.4 logs. The largest decline (3.0 log CFU/ml), where *Salmonella* populations were still detectable, was in manganese. *Salmonella* populations decreased by 3.1 logs in groundwater during the 7 day storage at 30 °C. The largest decline (4.0 log CFU/ml), where *Salmonella* populations were still detectable, was in Solubor.

Significance: These results demonstrate that some fertilizers may support *Salmonella* survival over extended periods if left in spray tanks and may be a source for pathogen contamination in foliar application in produce fields.

P2-50 Control and Prevention of Microbial Hazards in Greenhouse Tomatoes: Integrated Food Safety and Plant Health Approach

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Developing Scientist Competitor

Introduction: Tomatoes have been linked to multiple outbreaks of foodborne disease. In addition, tomato diseases are ranked the highest risk to greenhouse tomato productivity due to their destructiveness and the lack of effective management strategies.

Purpose: Systems approaches that integrate prevention and control of human and plant pathogens may provide a comprehensive, successful strategy to minimize food safety risks and achieve high quality product.

Methods: To address this, a multidisciplinary team of food safety experts and plant pathologists performed on-site surveys to identify greenhouse production methods and practices used by industry in US, Canada and Mexico. Standardized, pre-test questionnaires were used to assess the practices. Expert stakeholder groups performed impact analysis. Risks were ranked for *Salmonella* spp., *E. coli*, *Listeria monocytogenes*, *Clavibacter michiganensis* subsp. *michiganensis*, *B. cinerea*, Pepino mosaic virus and emerging tomato viroids.

Results: Tomato greenhouse production process flow diagrams were constructed for large/medium/small growers that included a total of 293 practices performed during propagation, growing and post-harvest stages of production. Points of pathogen entry, dissemination and proliferation were identified throughout the seed-to-retail production cycle. The results were merged into operational risk assessment profiles and high risk practices were identified. Identified points critical for simultaneous control of human and plant pathogens differed between large/medium/small operations. While interventions targeting quality of irrigation water and inter-planting have the highest impact in large scale greenhouses, priorities for small greenhouses were identified in harvest and post-harvest stages of production.

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Significance: Development of a system-wide framework in which the introduction and spread of plant and human pathogens can be effectively managed will enhance public health and provide the fundamental basis for growth of the greenhouse industry.

P2-51 The Impact of Shell Damage and Paracetic Acid on Microbial Loads of Harvested and Hulled Walnuts

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Developing Scientist Competitor

Introduction: At harvest, walnuts are mechanically shaken to the orchard floor, collected and transported to a hulling facility where the outer hull is removed by abrasion and water rinse. Shells can be damaged in this process potentially exposing the kernel to various degrees of microbial risk.

Purpose: To determine the impact of shell damage and peracetic acid (PAA) treatment on the microbial loads of walnuts collected at a commercial hulling facility.

Methods: Inshell Chandler variety walnuts were collected from the tree canopy, before (at receipt), during (from the float tank), and after (from the sort line) hulling, after drying, and at monthly intervals during ambient storage. Immediately before the sort line, walnuts were rinsed with either water or 200 ppm PAA. Inshell walnuts were shaken for 2 min in buffer; kernels extracted from walnuts with visibly intact or damaged shells were stomached for 30 s in buffer. Samples were plated onto tryptic soy agar with cyclohexamide or Chromagar ECC to determine aerobic plate (APC) or coliform (CC) counts, respectively.

Results: Levels of APC and CC on inshell walnuts were 5 and 4 log CFU/nut, respectively, from the tree samples. These levels increased and then decreased approximately 1 log CFU/nut during hulling and drying, respectively; levels further declined during storage. Microbial levels on kernels extracted from preharvest walnuts were at the limit of detection (20 CFU/nut), increased by 1 to 2 log CFU/nut during hulling, and declined after drying and during storage. Consistently, but not always significantly, higher counts were observed on kernels extracted from walnuts with broken shells. Counts on PAA-treated walnuts (both inshell and kernels) were not significantly lower than those treated with water.

Significance: Microbial levels on walnut kernels increase during hulling regardless of shell damage; higher populations are associated with shell breakage.

P2-52 The Effects of Integrated Treatment of UV Light and Low-dose Gamma Radiation on *Escherichia coli* O157:H7 and *Salmonella enterica* on Grape Tomatoes

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Introduction: Foodborne disease is increasing worldwide, with millions of cases occurring each year. In recent years considerable numbers of foodborne disease outbreaks associated with produce were reported and specifically tomatoes have been involved with a number of multistate outbreaks.

Purpose: The purpose of this study was to evaluate efficacy of integrated treatment of UVC and low dose Gamma radiation to inactivate inoculated mix strains of *Escherichia coli* O157:H7 and *Salmonella enterica* on whole grape tomatoes.

Methods: A mix bacterial cocktail composed of a three-strain mixture of *E. coli* O157:H7 (C9490, E02128 and F00475) and a three-serotype mixture of *S. enterica* (*S.* Montevideo G4639, *S.* Newport H1275, and *S.* Stanley H0558) were used for this study. Selection of these strains was based on their association with produce-related outbreaks. Spot inoculation (50-100 microliter) of tomatoes (surface and stem sites) was performed to achieve population of appropriately 10^{7-8}CFU/tomato. Inoculated tomatoes were subjected to UVC (253.7 nm) dose of 0.6 KJ/M^2 followed by four different low doses of gamma radiations (0.1 kGy, 0.25 kGy, 0.5 kGy, 0.75 kGy). The quality aspect (lycopene content, color, and texture) and the fate of background microflora (mesophilic, psychrotrophic, mold and yeast counts) during storage at room temperature (~ 22 °C, 60% RH) over 21 days were also determined.

Results: Integrated treatment with UVC light plus low dose radiation significantly ($P < 0.05$) reduced the population of the target pathogens on whole grape tomato. Results indicate about 3.4 ± 0.27 and 3.0 ± 0.12 log CFU reduction of*E. coli* O157:H7 and *S. enterica* per tomato was achieved with UVC treatment (0.6 KJ/M^2) combined with 0.25 kGy radiation. Even better, more than a 5 or 6 log CFU reduction per fruit was accomplished by combining UVC treatment with 0.5 kGy and 0.75 kGy radiation, respectively, for all tested pathogens. Furthermore, UVC light treatment with Gamma radiation significantly ($P < 0.05$) reduced the native microflora on tomatoes compared to control during storage.

Significance: Data obtained from this work suggests efficacious treatment strategy for produce indicating 5 or higher log reduction which is consistent with the recommendations of the National Advisory Committee on Microbiological Criteria for Foods.

P2-53 A Baseline Analysis of Washington State Fresh Market Apple Packinghouse Food Safety Practices Relating to Microbial Hazards

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Introduction: The Washington fresh market apple industry has an excellent record of providing safe apples to consumers. Yet there is limited research as to the effectiveness of various practices used to mitigate microbial hazards on apples; specifically relating to the occurrence of pathogens on the fruit itself or in packinghouses that pack whole fresh market apples.

Purpose: This research documents the current food safety programs and mitigation practices that the fresh market apple packing companies in the state of Washington are using to address potential microbial hazards.

Methods: The study methodology included: 1) the development and delivery of a survey instrument containing questions on source water types and testing, process water testing, environmental testing frequency and location, food safety programs utilized, and microbial pathogen mitigation measures, 2) the collection of microbial testing data, creation of a database and analysis of the data for variations and trends, and 3) a review of current mitigation practices.

Results: Fifty-five percent of all conventional and organic apple packing companies in Washington responded to the survey. Subsequently, 29% of all companies in the industry provided their 2005-2010 water, environmental, and product microbial test data. The data was compiled and analyzed for trends. Water testing is more prevalent than other types of testing. Environmental testing has generally increased since 2006 while product testing has decreased since 2008.

Significance: The results of both the survey and test data provide apple packinghouses with baseline information about practices in their industry which is especially beneficial for quality process validation, continuous learning and ultimately quality improvements.

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P2-54 Hard and Semi-hard Natural Cheese Slices Do Not Support Growth of *Salmonella* spp. during Storage at 25°C

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Developing Scientist Competitor

Introduction: Food regulatory and industry personnel have long questioned whether cheese can be safely stored at non-refrigeration temperatures. Research is needed to determine whether cheese would support the growth of pathogens when stored under conditions similar to a retail display.

Purpose: The purpose of this study was to investigate the post-processing behavior of *Salmonella spp.* when inoculated onto hard and semi-hard natural cheeses with elevated temperature storage.

Methods: Ten varieties of commercial, pre-sliced natural cheeses were purchased from the supermarket. Slices (20 g) were surface inoculated with 100 µl of a 6-strain cocktail of 10^7 log CFU/mL *Salmonella spp.* using an L-shaped spreader. Slices were air dried under a hood, then folded to enclose the inoculum and placed into standard cheese bags. Bags were vacuum sealed and stored aerobically at 25 °C up to 16 days, with samples taken every 2-3 days. At each sampling point, a slice was diluted 1:9 with Butterfield's Phosphate Diluent and stomached 2 min. Samples were serially diluted in BPD and plated on Modified Eosin Methylene Blue agar. Plates were incubated at 37 °C for 24 h, after which counts were taken and log CFU/g was calculated for each sample. Duplicate trials were completed for all cheeses. Death curves for each cheese were fit linearly using Combase DMFit software.

Results: Death rates ranged from –0.07 to –0.01 log CFU/g per day, representing Swiss and reduced-sodium Provolone cheeses, respectively, with total reduction over storage ranging from –1.2 to –0.3 log CFU/g, again in Swiss and reduced-sodium Provolone. Change in sample pH over storage ranged from –0.55 in Provolone to +0.08 in Colby cheese.

Significance: This research confirmed that *Salmonella spp.*, when introduced post processing, is unable to grow at 25 °C in the following cheese slices: Cheddar (mild, medium, and sharp), Colby, Gouda, Havarti, Muenster, Provolone, reduced-sodium Colby Jack, and reduced-sodium Provolone. In conjunction with cheese challenge studies investigating behavior of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7, this data will be significant in identifying cheeses safe for unrefrigerated storage.

P2-55 Microbiological Profile of Two Artisanal Mexican Cheeses During Manufacturing Process

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Introduction: Artisanal Mexican cheeses generally are made by farmers on a small scale using traditional techniques and in some occasions raw milk. The sensorial characteristics are unique, however the final quality of the product, including safety, is variable and could represent a health risk to the consumer.

Purpose: The objective of the present study was to study the evolution of the microflora during the processing of two Mexican artisanal cheeses ("bola" and "poro") produced during dry and rainy seasons.

Methods: Along the manufacturing process, samples of bola and poro cheese were analyzed to quantify the levels of aerobic plate count (APC), lactic acid bacteria (LAB), yeast, molds, coliforms, and *Escherichia coli*. The presence of *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella* spp. were also determined.

Results: A total of 107 and 143 samples of bola and poro cheese were analyzed. In bola cheese along the ripening (50, 80, and 110 days) populations of all microbial groups decreased; the levels of APC, LAB, yeasts and molds ranged from 7.2 to 5.6, 7.6 to 6.0, 3.9 to 1.4 and 4.5 to 3.6 log CFU/g, respectively. In poro cheese after 7, 30 and 60 days of storage, populations of APC, LAB, yeast and molds ranged from 6.1 to 5.8, 6.2 to 5.9, 4.0 to 3.4 and 4.4 to 4.2 log CFU/g, respectively. In general in both type of cheeses produced during rainy season, the levels of all indicator microorganism were higher. In all samples coliforms and *E. coli* were under detection limit (<3 MPN/g), whereas *L. monocytogenes, S. aureus* and*Salmonella* were not isolated from any sample.

Significance: These results showed that microbial quality of artisanal cheeses is acceptable. However, if the manufacturing method is not standardized the risk for the consumer still is present.

P2-56 Antimicrobial Residues in Raw Milk Produced in Minas Gerais State, Brazil

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Introduction: Surveillance of antimicrobial residues in milk is a frequent concern for several countries, including Brazil, because they can affect food safety, the quality of dairy products, and also the environment.

Purpose: The aim of this work was to evaluate the presence of veterinary drugs in 132 Brazilian raw milk samples collected from bulk tanks in Minas Gerais state.

Methods: Milk samples submitted to analysis of 30 analytes by liquid chromatography coupled to mass spectrometry *in tandem*. The antimicrobial groups, analytes, and MRL (Maximum Residue Limit) according to Brazilian legislation (expressed in µg/kg) were: a) Beta-lactam: Penicillin G and V (4.0/each), Ceftiofur (100.0), Cloxacillin, Oxacillin, and Dicloxacillin (30.0/each); b) Tetracyclines: Chlortetracycline, Doxycycline, Tetracycline and Oxytetracycline (maximum value 100.0); c) (Fluor) Quinolones: Oxolinic acid, Nalidixic acid, Norfloxacin, Sarafloxacin, and Difloxacin (not described); Flumequine (50.0), Ciprofloxacin, and Enrofloxacin (100.0/each); d) Sulfonamides: Sulfadimethoxine, Sulfaquinoxaline, Sulfadiazine, Sulfathiazole, Sulfapyridine, Sulfamethoxazole, Sulfamethazine, Sulfachlorpiridazine, Sulfizoxazole, Sulfadoxine, and Sulfamerazine (maximum value 100.0) and Trimethoprim (not described).

Results: Residues of Penicilin V (0.76%), Ceftiofur (5.30%), Oxacillin (1.52%), Dicloxacillin (1.52%), Oxytetracycline (2.27%), Ciprofloxacin (2.27%), and Enrofloxacin (2.27%) were detected. Only Penicillin V was detected in concentrations above the MRL (almost two folds) in all positive milk samples with the value of 7.95 µg/kg. The presence of these drugs in milk may be associated with treatments of infectious diseases in dairy cattle, mainly mastitis.

Significance: Although residues of antimicrobials have been detected in raw milk, the frequency of samples with values above the MRL was low, indicating that the these drugs have not been used in an abusive manner. The monitoring of these residues is important and must be done systematically to keep the food safety and protect the consumers.

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P2-57 Residues of Pesticides in Raw Milk from Minas Gerais State - Brazil

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Introduction: Residues of pesticides when present in milk can cause problems to public health and to environment. Few studies have been carried out in Brazil investigating the presence of these drugs in dairy products, despite of the worldwide awareness of their importance to public health.

Purpose: The aim of this study was to detect pesticide residues in 132 Brazilian raw milk samples collected from bulk tanks in dairy farms of Minas Gerais state, Brazil.

Methods: Raw milk samples were submitted to detection of 12 analytes for each sample, including drugs used to control endo and ectoparasites in cattle. Avermectins were analyzed by liquid chromatography coupled to mass spectrometry *in tandem* and Pyrethroids by gas chromatography with electron capture detection. The pesticides groups and analytes with Maximum Residue Limit based on Brazilian threshold (µg/kg) were: a) Avermectins: Abamectin (10.0), Doramectin (15.0), Eprinomectin (20.0), Ivermectin, and Moxidectin (10.0/each); b) Pyrethroids: γ-Cyhalothrin and λ-Cyalothrin (25.0/each), Cyfluthrin, Cypermethrin, Deltamethrin (20.0/each), Fenvalerate (40.0), and Permethrin (50.0).

Results: It was noted that 101 samples (76.51%) were negative for all the analytes and although 12 (9.09%) showed positive to at least one analyte, 93.18% of the samples were in agreement with Brazilian legislation. Although residues of Abamectin (5.30%), Doramectin (2.27%), Ivermectin (7.58%), Cypermethrin (9.09%), and Deltamethrin (1.52%) have been detected, only Cypermethrin was detected above the Brazilian MRL (6.82%) with concentrations up to 12.55 times above the MRL (20.0 µg/kg). Considering the legal values described by *Codex Alimentarius* and by countries such as United States of America and Japan (50.0 µg/kg), it can be noted that 3.03% of the samples showed values above these thresholds.

Significance: It can be concluded that Cypermethrin is the main pesticide found in raw milk from Minas Gerais State and since it represents a risk to public health, corrective actions must be adopted by dairy farms to avoid milk contamination and problems for consumers.

P2-58 Comparing the Behavior of Multidrug-resistant and Pan-susceptible *Salmonella* during the Manufacture and Aging of a Semi-hard Cheese Manufactured from Raw Milk

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Introduction: Numerous outbreaks of salmonellosis have been linked to the consumption of cheese. There is concern that emerging multidrug-resistant strains of *Salmonella* may exhibit increased virulence and tolerance to stresses encountered in food production compared to less resistant strains, which may enhance survival in cheese.

Purpose: This study compares the behavior of multidrug-resistant and pan-susceptible *Salmonella* during the manufacture and aging of Gouda cheese and to compare current enrichment and detection protocols.

Methods: Cheeses were manufactured, in triplicate, in a lab scale cheese vat from raw milk inoculated with a 6-strain cocktail of either resistant or susceptible *S.* Newport and *S.* Typhimirium at an approximate concentration of 20 CFU/ml. Samples of milk, whey, curd and finished cheese throughout aging were analyzed using each of the following enrichment protocols: ISO 6759:2002; AOAC-RI for BAX PCR; modified versions of AFNOR (BAX) and Health Canada MFLP-29. All results were culture confirmed.

Results: Overall, *Salmonella* counts increased significantly during manufacture to a mean count of 734 CFU/g of cheese on day one followed by a significant decrease over 60 days aging to a mean of <1 CFU/g. Levels fell and stayed below the direct plating detection limit of ≥5 CFU/g after 54 days on average, yet remained detectable following enrichment for 210 ± 40 days. Changes in pathogen levels observed throughout manufacture and aging did not differ significantly between cheeses inoculated with resistant or susceptible *Salmonella*. The ISO method with and without PCR detection produced the least false negative results followed by AOAC-RI for BAX. The remaining methods, notably those with elevated primary incubation temperatures, produced results that disagreed significantly with the true result according to McNemars test.

Significance: Our results suggest that while multidrug-resistant strains of *Salmonella* may not display increased survivability in Gouda cheese, the 60-day aging requirement alone is insufficient to completely eliminate viable levels of this pathogen.

P2-59 Bacteriocinogenic and Virulence Potential of *Enterococcus* spp. Isolated from Raw Milk and Cheese

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Introduction: Besides playing an essential role in the development of the organoleptic characteristics of dairy products, enterococci also contribute to their safety due to production of antimicrobial peptides, such as bacteriocins. However, enterococci have emerged as serious pathogens in nosocomial infections.

Purpose: This study focused on the molecular and phenotypical characterization of *Enterococcus* isolates obtained from raw milk and cheese, aiming the determination of their bacteriocinogenic activity and virulence potential.

Methods: Forty-three bacteriocinogenic enterococci isolates, obtained from raw milk and cheese and identified by 16s rDNA, were fingerprinted by RAPD-PCR analysis, and tested for the presence of genes for lantibiotics (*lan*M, *lan*B and*lan*C) and enterocins (*ent*A, *ent*B, *ent*P, *ent*L50AB and *ent*AS48) by PCR. Isolates were also tested for bacteriocins production and inhibitory spectrum using phenotypical tests. Also, the virulence potential of the isolates was evaluated by investigation of genes *gel*E, *hyl*, *asa*1, *esp*, *cyl*A, *efa*A, *ace*, *van*A, *van*B, *hdc*1, *hdc*2, *tdc*, and *odc* and by testing the capability to produce gelatinase, lipase, DNAse and α and β-hemolysis.

Results: RAPD analysis divided the 43 isolates in 20 groups (I to XX), being groups III and IV those that contained more isolates (9 and 6, respectively). The antimicrobial substances produced by most isolates were sensitive to α-chymotrypsin, proteinase K, and trypsin, indicating their proteinaceous nature. The isolates presented antimicrobial activity against most *Listeria* spp. Among the 21 isolates that contained genes for lantibiotics, 14 contained only *lan*B, 3 contained only *lan*C, 3 contained *lan*B and *lan*C, and 1 contained *lan*B and *lan*M. Genes for enterocins P, A and AS48 were detected in isolates from 11, 6 and 5 different RAPD groups. Most RAPD groups contained isolates positive for the tested virulence genes, mainly *asa*1 (100%), *gel*E (93.0%), and *efa*A (83.7%). 53.5% of the isolates presented β-hemolysis.

Significance: The study demonstrated the contradictory characteristics of the tested *Enterococcus* isolates: they presented a good potential for application in food biopreservation but contained several virulence factors.

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P2-60 Inactivation of *Listeria monocytogenes* and Shiga Toxin-producing *Escherichia coli* in Low-sodium Cheddar Cheese Extract Supplemented with Natural Fermentates and Adjunct Cultures

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Developing Scientist Competitor

Introduction: Salt is a natural preservative in cheese. Production of low-sodium cheese will require modified make procedures or adjunct ingredients compared with traditional cheeses to ensure quality and safety.

Purpose: To identify natural fermentates or protection cultures to enhance the inactivation of *Listeria monocytogenes* (Lm) and Shiga toxin-producing *E. coli* (STEC) in a model cheese system.

Methods: Low-salt Cheddar aqueous extract (CCE; adjusted to 1.89% salt-in-moisture, 2.7% lactate-in-moisture, pH 5.6) was supplemented with commercial fermentates (0.1% or 0.2% Fermentate B, 0.5% or 1.0% Fermentate A or C) or 5-log CFU/ml of protective cultures X, Y, or Z (*Bifidobacteria, Lactobacillus*, or *Lactococcus*). In addition, a Control without additives was tested. Treatments were inoculated with 3.5-log CFU/ml Lm or 5-log CFU/ml STEC (3-strain mixtures for each inoculum type) and triplicate samples assayed weekly for changes in microbial populations during 60 days storage at 10 °C. Each study was replicated twice.

Results: STEC survived longer than Lm in Control CCE stored 60 days at 10 °C; populations of STEC decreased an average 1 log in 60 days and Lm decreased 3 logs in 42 days. None of the fermentates changed the inactivation rate of STEC, but populations of Lm decreased 3.3 and 2.9 log at D-7 for 1.0 and 0.5% Fermentates A, respectively, and 1.8 log decrease for 1.0% Fermentate C. STEC populations declined 2 and >5 log at D-42 and 60 in CCE supplemented with Culture Z, respectively, and Lm declined 3 logs in 28 days. Cultures X and Y had no significant effect on the inactivation of STEC or Lm compared to the Control.

Significance: This study identified fermentates and protective cultures to enhance the inactivation of Lm and STEC in a model low-sodium cheese system. Additional studies should confirm efficacy in natural cheeses such as Cheddar and Mozzarella.

P2-61 Evaluation of Compositional Factors of Low-sodium Cheddar Cheeses on the Growth of Pathogens in a Model System

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Developing Scientist Competitor

Introduction: The microbial safety of cheese results from the combined activity of pH, NaCl, organic acids, competitive microbiota, and moisture content, as well as the controlled temperatures used during ripening. Recently, there is significant interest in manufacturing low-sodium, low-fat Cheddar varieties; these varieties necessitate the use of modified make procedures and composition that differ significantly from traditional Cheddar.

Purpose: To determine the level of lactic acid, salt and pH to inactivate four different foodborne pathogens in a model low-sodium Cheddar cheese extract.

Methods: The Cheddar cheese model system employed a water extract of Cheddar cheese (CCE) as the medium. For Phase 1, CCE [1.89% salt-in-moisture (S/M); 2.7% lactate-in-moisture (L/M); pH 5.4] representing the most permissive conditions in low-sodium Cheddar, was inoculated with 4 log CFU/ml *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* or Shiga toxin-producing *Escherichia coli* (STEC) (3 strain mixtures of each). For Phase 2, eight CCE treatments (full-factorial design; 1.2 and 4.8% SIM; 2.7 and 4.3% LIM, pH 4.8 and 5.3) were inoculated with STEC. For both studies, treatments were dispensed into vials, and triplicate samples assayed weekly during six weeks storage at 11 °C. Each study was replicated twice.

Results: In the first study, STEC declined 0.4 log in the CCE, whereas populations of the other pathogens decreased an average 1.1 to 1.9 log during the 6 week storage. For the second study, STEC declined most rapidly in CCE with lower pH (4.8) and higher protonated lactic acid concentrations, whereas salt concentration (1.2 vs. 4.8%) did not have a significant effect on survival during the 42-day storage at 11 °C.

Significance: These results confirm that initial pH of cheese is essential in safety, regardless of salt content; this study will provide the cheese industry the levels of critical factors that must be present to produce a safe, low-sodium cheese.

P2-62 Free Amino Acids and Biogenic Amines in High-pressure Treated Blue Cheese

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Introduction: Highly proteolyzed cheeses, usually of long ripening periods, are rich in free amino acids (FAA), which may be converted into biogenic amines with the subsequent risk for consumers. Blue cheeses, because of the presence of fungal proteinases and peptidases, fall within this group.

Purpose: The objective of the present work was to investigate the formation of biogenic amines in commercial blue cheese, and to study the prevention of biogenic amine formation by means of cheese pressurization.

Methods: Two batches of commercial blue cheese, made from pasteurized ewe's milk, were investigated for the formation of FAA and biogenic amines during a 360-day ripening period. Cheeses from each batch were pressurized, at 400 or 600 MPa, on days 21, 42 or 63 of ripening. Unpressurized cheeses served as control. FAA and biogenic amines were determined as previously described.

Results: FAA concentration declined significantly with cheese pressurization. After 360 days, control cheese contained 11,676 mg FAA/kg cheese DM. Cheeses pressurized at 400 MPa on days 21, 42 or 63 contained at that time 8,145, 11,668 and 12,347 mg FAA/kg cheese DM, respectively, whereas cheeses pressurized at 600 MPa on days 21, 42 or 63 contained only 5,433, 9,580 and 9,481 mg FAA/kg cheese DM, respectively. Tyramine reached 52 mg/kg DM in 360-day-old control cheese. Cheeses pressurized at 400 MPa on days 21, 42 or 63 contained at that time only 32.0, 20.9 and 20.6 mg tyramine/kg DM, respectively, whereas cheeses pressurized at 600 MPa on days 21, 42 or 63 contained 33.4, 32.4 and 27.2 mg tyramine/kg DM, respectively.

Significance: Formation of biogenic amines in blue cheese was influenced by pressurization, which lowered tyramine concentration.

P2-63 Optimizing Bulk Milk Dioxin Monitoring Based on Costs and Effectiveness

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Introduction: Dioxins are environmental pollutants present in the agri-food chains. The negative consequences associated with their presence in food are not only related to human health but also to the food businesses operators embedded in the food chains. Food and feed dioxin-monitoring programs aiming to detect, control and reduce the presence of dioxins in food chains have been implemented. However, to date, the costs and effectiveness of such programs have not been assessed.

Purpose: This study aims to quantify the costs and effectiveness of bulk milk dioxin monitoring to optimize the sampling and pooling strategies for a selected set of contamination scenarios.

Methods: Two different optimization models were built using a linear programming methodology. The first model aims to minimize the monitoring costs subject to a minimum required effectiveness, while the second model aims to maximize the effectiveness of monitoring for a given monitoring budget.

Results: The study shows that a higher level of effectiveness is possible but at higher costs. Monitoring programs with 95% effectiveness aiming to detect a single contaminated farm with a tank milk concentration equal to the EC legal action limit (i.e., 2 pg TEQ/g fat) would cost €2.6 million per month. A large reduction in monitoring costs (at the same level of effectiveness) is possible at intermediate incident sizes (i.e., 73% reduction when two farms are contaminated at 3 pg TEQ/g fat), which is close to the smallest incident aimed to be detected.

Significance: Both models enable the analysis of the costs and the effectiveness of bulk milk dioxin monitoring programs, offering quantitative support to risk managers of the food industry and the food safety authority. Additionally, this study proves that the effectiveness of monitoring depends not only on the performance of the detection tests but also on the number of samples collected.

P2-64 Survival of Lactic Acid Bacteria with Probiotic Potential during Shelf Life of Fermented Green Olives under Modified Atmospheres

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Introduction: Fermented foods can be characterized as probiotic foods if they contain an appropriate amount of viable and active probiotic microorganisms (10^6-10^7 CFU/g) enough to reach the intestine and exert an equilibrating action on the intestinal microbiota.

Purpose: To evaluate the ability of lactic acid bacteria with probiotic properties used as starters in olive fermentation to retain adequate populations during the shelf life of fermented green table olives under modified atmospheres.

Methods: Samples of 100 g of fermented green olives cv. Halkidiki, were packed in polyethylene pouches in modified atmospheres (70% N_2:30% CO_2) and stored at 4 and 20 °C for 12 months. The experiment consisted of four packaging treatments with olives previously fermented by: indigenous microbiota (C), *Lactobacillus pentosus* (A), *Lactobacillus plantarum* (B), and mixture of both strains (S). The inoculated strains have been previously selected for retaining *in vitro* probiotic potential. Microbiological (lactic acid bacteria-LAB, yeasts/molds), physicochemical (pH, acidity, salt content, color, firmness) and sensory analyses were conducted throughout storage. The survival and the variability of the LAB strains were assessed by pulsed field gel electrophoresis (PFGE).

Results: The population of LAB at the beginning of storage was 5.5-5.9 log CFU/g. At the end of storage period, the population of LAB at 4 °C decreased up to 0.5 log CFU/g, depending on the case, whilst at 20 °C increased approximately 1 log CFU/g in the cases A and S, 0.5 log CFU/g in B and remained stable in C. According to the PFGE, the survival of the inoculated strains was higher for *Lb. pentosus*, depending on the case and the storage temperature. According to the sensory and physicochemical analyses, the quality characteristics of the olives were better preserved at 20 °C.

Significance: Fermented green olives with lactic acid bacteria exhibiting probiotic potential were found to contain adequate amounts (10^6-10^7 CFU/g) of the inoculated starters even after a 12 month storage, retaining desirable sensory characteristics.

This study was funded by the EU project PROBIOLIVES (FP7-SME-2008-2-243471)

P2-65 Poor GMPs Lead to a Second Occurrence of Staphylococcal Food Poisoning

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Introduction: Over 100 individuals were sickened after ingesting an assortment of desserts linked to four staphylococcal food poisoning outbreaks leading investigators to products manufactured by an Illinois bakery. Less than a year later the same bakery was implicated in a second staphylococcal food poisoning outbreak involving the firm's Black and White Chiffon Mousse Cake served at a birthday party with 16 complaints of illness. This study examines association and pathogenic potential of the isolates recovered from the environmental and product samples obtained during multiple inspections of this firm.

Purpose: Investigation of a firm implicated in reoccurring incidences of staphylococcal food poisoning episodes.

Methods: *S. aureus* isolates were evaluated using either commercially available serological kits or a multiplex PCR method for the identification of staphylococcal enterotoxins SEA-SEE, SEG-SEI, SE-like SEIJ-SEIU or their respective genes and for Panton-Valentine leukocidin (PVL) cytotoxin. Antimicrobial susceptibility testing was performed using the VITEK 2 AST-CP67 Gram Positive Susceptibility Card, an *in vitro* method used in clinical laboratories for antimicrobial testing. PFGE subtyping method was performed for the 85 *S. aureus* isolates digested with the primary enzyme *Sma*I.

Results: FDA's investigative team visited the bakery several times and identified substantial deviations from the current Good Manufacturing Practice Regulations monitored by their Code of Federal Regulations 21 CFR. A concentrated whipped topping ingredient and 12% of the environmental samples revealed the existence of enterotoxigenic *S. aureus* isolates capable of producing diverse combinations of toxins. Additionally, antimicrobial susceptibility testing found that all *S. aureus* isolates were resistant to one or more agent(s). PFGE characterization identified thirteen pattern types from the bakery's environment and the raw ingredient sample.

Significance: The significant observations resulting from this investigation demonstrates the prevalence and diversity of antimicrobial resistant strains of *S. aureus* carrying of both classical SE's and genes for non-classical SE and SE-like enterotoxins found in the environment.

P2-66 *In Vitro* Probiotic Potential of Lactic Acid Bacteria Isolated from Minas Artisanal Cheese Produced in Serra da Canastra, Brazil

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Introduction: In Brazil, artisanal cheeses made of raw milk have social, economic, and cultural importance. Lactic acid bacteria (LAB) are the main component of their desirable microbiota and may play important roles such as development of flavor and protection against undesirable bacteria.

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Purpose: This work aimed to select LAB previously isolated from Minas artisanal cheeses produced in Serra da Canastra, Brazil, according to their probiotic properties to produce novel foods in the future.

Methods: The probiotic profile of 12 samples of LAB (11 *Lactobacillus* spp. and one *Weissella paramesenteroides*) isolated from artisanal cheeses and identified by PCR-ARDRA 16S-23S was *in vitro* evaluated by antimicrobial susceptibility using agar diffusion disk test, antagonism against pathogens and LAB, and tolerance to gastric pH and biliary salts. All tests were carried out in triplicate, with three repetitions and the means were compared by the Kruskal-Wallis test ($P < 0.05$).

Results: All LAB were sensitive to clindamycin, erythromycin, and tetracycline and resistant to vancomycin. *L. rhamnosus* B4, *W. paramesenteroides* C10, and *L. rhamnosus* D1 showed resistance to fewer antimicrobials (ceftazidime, oxaciclin, streptomycin, and vancomycin). All LAB showed higher inhibition halos ($P < 0.05$) against pathogens than against other LAB isolated from the same cheeses. Regarding gastric pH tolerance, all samples showed low inhibition of growth at pH 2. The tolerance to biliary salts varied from low to high among the tested samples.

Significance: *L. rhamnosus* B4, *W. paramesenteroides* C10, and *L. rhamnosus* D1 showed the best probiotic potential and may be used to improve the sanitary quality of artisanal cheeses preserving their microbial identity.

P2-67 Pickled Egg Production: Effect of Brine Acetic Acid Concentration, Brine Fill Temperature, and Post-packing Temperature on Acidification Rate

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Introduction: United States Federal Regulations require that acidified foods such as pickled eggs should reach a pH of 4.6 or lower within 24 hours of packaging or be kept refrigerated until then. Processes and formulations should be designed to satisfy this requirement, unless proper studies demonstrate the safety of other conditions.

Purpose: Our objective was to determine the effect of brine acetic acid concentration and packing conditions on the acidification rate of hard boiled eggs.

Methods: Hard boiled eggs were acidified (9 eggs per 32 oz glass jar) at various conditions (brine temperature / heat treatment of filled jars / post-packing temperature): (a) 25 °C / none / 25 °C, (b) 25 °C / none / 2 °C, (c) 85 °C / none / 25 °C, (d) 25 °C, 100 °C for 16 min / 25 °C. Three brine concentrations were evaluated (7.5, 4.9, and 2.5% acetic acid (AA)) and egg pH values (whole, yolk, 4 points within egg) were measured from 4 to 144 h, with eggs equilibrating at pH 3.8, 4.0, and 4.3, respectively. Experiments were conducted in triplicate. Multiple linear regression analysis was conducted to evaluate the effect on pH values at the center of the yolk.

Results: Regression analysis ($R^2 = 0.94$) showed no significant interaction between packing conditions and time ($P > 0.05$). Only the eggs that were acidified and kept refrigerated showed a significantly slower time to reach pH 4.6 ($P < 0.0001$). The interaction between brine concentration and acidification time was significant ($P < 0.0001$). Higher concentrations of brine decreased egg pH at a faster rate. When using linear regression to predict the time for the yolk center to reach pH 4.6, values ranged from 23.5 h for 7.5% AA brine to 220.3 h for 2.5% AA brine.

Significance: These results demonstrate the importance of conducting acidification studies with proper pH measurements to determine safe conditions to manufacture commercially stable pickled eggs.

P2-68 Antagonism of *Lactobacillus* spp. Isolated from Minas Artisanal Cheese Produced in Serra da Canastra, Brazil, against *Staphylococcus* spp.

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Introduction: *Lactobacillus* is a desirable lactic acid bacteria (LAB) found in a great variety of artisanal cheeses in which they play important roles as development of typical flavor and production of substances that reduce or inhibit the growth of pathogens. *Staphylococcus* is also found in artisanal cheeses and may elaborate enterotoxins that cause food intoxication.

Purpose: This study aimed to evaluate the inhibitory capacity of *Lactobacillus* spp. isolated from Minas artisanal cheese produced in Serra da Canastra, Brazil, against *Staphylococcus* spp. isolated from the same food.

Methods: Two samples of *L. rhamnosus* and one sample of *L. plantarum*, isolated from artisanal cheeses and identified by PCR ARDRA16S-23S, were submitted to *in vitro* antagonism test against 18 samples of *Staphylococcus* spp. isolated from the same cheeses. The analyses were carried out in triplicate considering three repetitions of the experiment. Means of inhibition halos were compared by the Kruskal-Wallis test ($P < 0.05$).

Results: All tested lactobacilli inhibited all samples of *Staphylococcus* spp. *L. plantarum* showed higher inhibition halos ($P < 0.05$) than the two samples of *L. rhamnosus* which presented similar mean inhibition halos ($P > 0.05$). These findings indicate that the inhibition behavior against *Staphylococcus* spp. may vary more between *Lactobacillus* species than between samples of the same species.

Significance: The *in vitro* antagonism of *Lactobacillus* spp. against *Staphylococcus* samples observed in this study may also occur in artisanal cheeses avoiding the production of staphylococcal enterotoxins and the occurrence of foodborne outbreaks related to the consumption of cheeses made of raw milk.

P2-69 Thermophilic Bacterial Populations from Raw Milk Differ from Those in Final Powder Products

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Introduction: Thermophilic bacilli are important spoilage bacteria in milk powder processing. An ongoing theory for the origin of such thermophiles in final powder product is from the raw milk, even though studies have suggested that thermophiles present in the final product originate from the manufacturing plant itself due to biofilm formation on processing surfaces.

Purpose: This preliminary study aimed to match thermophilic bacilli isolated from milk powders to those resident in the plant, and those coming in from the raw milk.

Methods: Bacterial isolates from the raw milk stream or powdered product were isolated at 55 °C and provided from a New Zealand manufacturing site. A total of 19 colonies were selected and identified using either species-specific PCR or the Microgen® Bacillus-ID and/ or the BBL™ Crystal Identification kits. RAPD PCR profiles for all isolates were also generated using primer ORP13. Following amplification, a cluster dendrogram was produced using the Pearsons correlation and UPGMA clustering in GelComparII.

Results: Overall, typical *Bacillus* isolates (e.g. *B. licheniformis*) were identified from the colonies from the raw milk stream, and RAPD analysis showed that they formed a genetic cluster, indicating species and strain similarities. Other thermophilic isolates (e.g. *Geobacillus* and *Anoxybacillus*) were identified from the evaporators and the powder sample, which also formed a second distinct genetic cluster by RAPD analysis. It seems probable that the isolates in the final powder product originated from the processing plant itself (*i.e.* the evaporators), and not from the raw milk stream.

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Significance: Routine testing of raw milk for thermophiles is an added cost for dairy manufacturers in New Zealand. A wider study confirming these preliminary results may allow a significant reduction in the costs of this type of testing.

P2-70 Characterization of the Lactic Acid Bacteria in Anbris (Fermented Goat's Milk) and Preliminary Probiotic Selection

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Introduction: Labneh Anbris is a traditional fermented cheese-like product made of goat's milk and highly consumed in many rural areas of Lebanon. The product is fermented over six months in a traditional setting without the addition of starter cultures, but dependent entirely on the natural microbiota present in the product. This natural fermentation process results in a final product with an extended shelf life at room temperature and contains good organoleptic characteristics.

Purpose: The purpose of this study was to investigate the potential probiotic lactic acid strains that may be naturally present in the product.

Methods: Samples were collected from two different villages (Arsal and Kweikh). Lactic acid bacteria were isolated on MRS Agar and Broth for *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* and M17 for the more fastidious*Streptococci* (incubated at 30° for 48 h). The isolated strains were then subjected to several biochemical and phenotypic tests to assess probiotic characteristics, namely, temperature and salt tolerance. NH_3 production, citrate utilization, acid and bile tolerance, antibacterial activity against *Staphylococcus aureus* and *ACE* inhibitory tests. Pulsed field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) were carried for isolates characterization.

Results: Results showed that six of the Gram-positive, catalase-negative, pooled isolates from Arsal and six isolates from Kweikh have potential probiotic characteristics by scoring positive to all the biochemical tests that were performed. Further molecular analyses and confirmation tests with PFGE and PCR indicated that the isolated probiotic strains are of the *Leuconostoc* (8 strains) and *Streptococci* (4 strains) genera.

Significance: The results are the first on such traditional products in Lebanon. It is highly important to identify probiotic strains in Anbaris. Anbaris is of high nutritional, health and economic value, and a commodity for isolating probiotic strains may be used in the production of new types of dairy-based functional foods.

P2-71 Fate of *Listeria innocua* in a Probiotic Dairy Dessert

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Introduction: Members of genus *Listeria* are able to survive to a wide range of stress conditions. *Listeria* is a ubiquitous microorganism, prevailing in raw materials and with known ability to persist in environments of processing, further contaminating processed foods. *Listeria innocua* has been used as a nonpathogenic surrogate for *L. monocytogenes* in survival and growth studies in foods.

Purpose: The objective of this study was to determine the fate of *L. innocua* in a probiotic dairy dessert supplemented with *Lactobacillus acidophilus* during chilling storage.

Methods: Three different formulations of dairy desserts were processed: F1 – control, inoculated with 10^4 CFU/g of *L. innocua* LH 475 only; F2 – added of 10^8 CFU/g of *L. acidophilus* only; and F3 – addition of both *L. acidophilus* (10^8 CFU/g) and *L. innocua* (10^4 CFU/g). Microbiological (*L. acidophilus* and *L. innocua*) and pH analysis were performed at time 0, 7, 14, 21 and 28 days of storage. *L. innocua* was enumerated using Oxford agar supplemented with antibiotics, while *L. acidophilus* was enumerated using MRS agar supplemented with 0.15% bile salts. Statistical difference was assessed through analysis of variance and Tukey's test using the XLSTAT for Window 2011.

Results: The pH of probiotic dairy desserts varied between 5.57 (F1) to 7.12 (F2) thoroughout the shelf life (28 days). Both time of storage and formulations significantly influenced the pH values ($P < 0.001$). The populations of *L. innocua*increased over the storage period independently of the formulation studied (F1 or F3) ($P < 0.001$). The populations of *L. acidophilus* increased expressively in F3 (from 7.04 to 9.01 log CFU/g), while in F2, the populations of this microorganism decreased from 7.78 to 6.59 log CFU/g.

Significance: It has been observed that *L. innocua* is not inhibited by the presence of probiotic culture in the dairy dessert.

P2-72 Behavior of *Listeria monocytogenes* in Dairy Products Contaminated Post-process

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Introduction: Following the adoption of the Food Safety Modernization Act, the FDA will be requiring specific actions for manufacturers of certain food products. Guidance for specific food products will likely be based on a risk assessment that will include the potential for *Listeria monocytogenes* (Lm) growth or survival in these products, should they become re-contaminated post process, prior to final packaging. Information on Lm growth is not available for many refrigerated dairy products.

Purpose: This study was conducted to determine the potential for Lm growth in a wide variety of dairy products during refrigerated storage.

Methods: Thirty-seven dairy products, including varieties of pasteurized process cheeses, cream cheeses, cultured aged cheeses, cultured mozzarella cheeses, feta cheese, cottage cheese and sour cream were inoculated with 100-500 CFU/g of a 6-strain cocktail of Lm and incubated at 45 °F for various time periods, depending on the shelf life of each product. Products were tested for Lm counts on modified Oxford Agar at varying intervals throughout the shelf life to determine if the organism was able to survive and grow in the products. Growth was considered an increase in counts of greater than or equal to 1 log level. The pH, water activity, fat, moisture, and salt of the uninoculated products were determined at the initiation of the study. Aerobic plate count testing was also performed using All Purpose Agar with Tween at the same test intervals throughout the study.

Results: The majority of products did not support the growth of Lm, and counts of the organisms decreased over the product shelf life in most cases. Only certain products were able to support the growth of Lm at refrigeration temperatures: certain pasteurized process cheeses and higher-pH aged cheese such as Gouda. Product formulation and presence of active cultures were important factors in determining the likelihood of Lm growth.

Significance: This information will be useful in determining the refrigerated dairy products having the potential to be contaminated post-process that are at risk for Lm growth during storage and distribution. The data can be used to determine which monitoring programs may be necessary in production facilities.

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P2-73 Survival of *Arcobacter butzleri* in Apple and Pear Purees

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Developing Scientist Competitor

Introduction: *Arcobacter* butzleri is considered potential zoonotic agents that is caused gastroenteritis in human and animal. However, inhibitory effects for *A*. butzleri are not investigated in natural compounds.

Purpose: The aim of this study was to examine the inhibition of *A.* butzleri in apple and pear purees.

Methods: The apple and pear purees were manufactured through advanced research. Three kinds of *A.* butzleri strains were spiked about 6.90-7.44 log CFU/ml in each purees. The number of bacteria, pH value and sugar contents were measured. All of the strains were decreased significantly each time point.

Results: *A.* butzleri were reduced about 7 log CFU/ml after 24-48 hours in both of the 4¡ÆC storage purees. *A.* butzleri CAU080180 strain was not detected in 12 hours and the others were not detected in 24-48 hours in 20¡ÆC storage purees. *A.* butzleri CAU076046, chicken intestinal isolate strain was most resistant but chicken skin isolate strain was sensitive in purees. There was no significant difference of the pH values and sugar contents in all of the groups.

Significance: *A.* butzleri was significantly inhibited in apple and pear purees. In further studies, other natural compounds like polyphenols should be evaluated to control new emerging pathogen, *A.* butzleri.

P2-74 Pilot Survey for Antimicrobial Resistance (AMR) in Bacteria from Australian Retail Foods

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Introduction: Raw retail foods destined for human consumption may harbor enteric bacteria. The presence of AMR in these organisms may indicate the development of resistance during food production or promote resistance in the human population.

Purpose: To determine the antimicrobial susceptibility of *Escherichia coli*, *Salmonella*, *Campylobacter* and *Enterococcus* in raw retail foods in Australia

Methods: Four retail foods: poultry, beef, pork and lettuce; along with four target organisms: *Campylobacter, Salmonella*, *E. coli* and *Enterococcus* were included in the survey. Nine food-bacteria combinations were chosen where the bacterial target was expected to exceed 10% in prevalence and to allow for the recovery of 100 isolates from each food-bacteria combination. Samples were collected from retail outlets in Sydney, Melbourne, Brisbane and Perth which accounts for the retail supply to 57% of Australians. Resistance to antimicrobials was determined in a central laboratory using Sensititre apparatus and CLSI breakpoints (where available).

Results: Isolation rates of the target organisms were sufficient to achieve the recovery of 100 isolates in seven of the nine food-bacteria combinations. Overall, resistance to the majority of antimicrobials was low by international comparison. Resistance to critically important antibiotics such as vancomycin, fluroquinolones and 3rd and 4th generation cephalosporins was non-existent or extremely low in the 300 *Enterococcus,* 299 *E. coli*, 100 *Salmonella* and 100 *Campylobacter* isolates tested. Ampicillin resistance in *E. coli* isolates from pork (28%) was the only resistance shown to be >10% higher than those observed in other international monitoring programs.

Significance: These data suggest that antimicrobial resistance in the selected food-bacteria combinations is generally very low. This data may be useful in managing and controlling AMR development in the Australian food chain and managing resistance in the community.

P2-75 Growth and Resistant Patterns of Various *Bacillus cereus* Isolates from Food-related Environments

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Developing Scientist Competitor

Introduction: *Bacillus cereus* spores are highly resistant to adverse treatments such as high temperature, radiation and chemicals. In addition, recent studies suggested that use of decontamination agents may increase antimicrobial resistance of foodborne pathogens.

Purpose: The objective of this study was to compare growth and resistant patterns of *Bacillus cereus* isolates obtained from different antimicrobial applications.

Methods: *B. cereus* isolates (S-BC-50 and S-BC-52: isolates from 10% NaClO application, S-BC-23 and S-BC-48: isolates from 0.5% H_2O_2 application, S-BC-54 and S-BC-56: isolates from 1.0% acetic acid application), and *B. cereus*KCTC1661 (standard strain) were inoculated into typtic soy broth (TSB), and incubated at 7, 10, 15, 25, 30 and 35 °C for comparison of growth pattern. To compare antimicrobial resistance of *B. cereus* isolates, exponential and stationary phase cells of *B. cereus* strains were subjected to TSB supplemented with NaClO (0-3.6%), H_2O_2 (0-0.28%) and acetic acid (0-1.5%) for 45-60 min, depending on the antimicrobials. Cell counts of *B. cereus* were enumerated on tryptic soy agar and the survival data of *B. cereus* were fitted to the simple linear regression to calculate death rates (log CFU/ml/min).

Results: Cell counts of all *B. cereus* strains decreased at 7 °C, but no differences ($P > 0.05$) of growth pattern were generally observed at 10-35 °C except for NaClO isolate (*B. cereus* S-BC-50 and S-BC-52) which had lower maximum cell density than other strains by 2 log CFU/ml at 10 °C. As *B. cereus* isolates were exposed to decontamination agents, exponential phase cells were more sensitive ($P < 0.05$) than those of stationary cells. Moreover, the differences in death rates (0.0-0.25 log CFU/ml/min) of isolates were not observed ($P > 0.05$) as subjected to the decontamination agents.

Significance: This result indicates that applications of decontamination agents may not cause different growth patterns and the resistance of *B. cereus* to the decontamination agents.

P2-76 Killing Efficiencies of *Salmonella* Bacteriophages and Survivability under Various Environmental Conditions

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Introduction: Phage-based therapies have great potential for a variety of antibacterial applications. We previously demonstrated that bacteriophages can be microencapsulated and directly fed to food animals to reduce foodborne pathogen transmission.

Purpose: Here we further characterized the *Salmonella* phages in our growing library in terms of growth kinetics and survivability under various conditions.

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Methods: Single-step growth kinetics were measured in *Salmonella* serovars Typhimurium, Kentucky and Enteritidis. Survivability was measured under varying pH, temperature, incubation with digestive enzymes, microwave and UV irradiation.

Results: There was a large amount of variability in bacteriophage growth rates (ranging from 0.0001 phage/cell/minute to 75 phage/cell/minute), adsorption rates (ranging from 0.54 phage/cell/minute to 0.99 phage/cell/minute), burst sizes (ranging from 0 to 4,567 particles) and latent periods (ranging from 21 min to 99 min). Overall, our phages had highest killing efficiencies in serovar Typhimurium. All phages showed lower killing efficiencies in serovar Kentucky. At pH 4.0, phage viability decreased by 0.5 log viable particles after 180 minutes. Similarly, phage viability decreased by 1.7 log viable particles at pH 10.0 after 180 minutes. Phages were significantly affected by acidic environments as there was a > 99.0% reduction in the number of viable particles at pH 2.0 after 5 min. Phages were unaffected by low concentrations of trypsin or chymotrypsin, but were quickly inactivated by incubation in 0.05% hypochlorite (chlorine is commonly used in drinking water in US poultry production). All phages were quickly inactivated by both microwave and UV irradiation (320 nm and 365 nm).

Significance: Thus, different phages possessed widely different killing efficiencies in vitro, but displayed more uniform survivability characteristics under different manufacturing conditions. Taken together, these data may help us predict other areas where phage-based therapies may be effective and appropriate as part of a biocontrol strategy.

P2-77 Thymol and Carvacrol Binary Mixtures to Control *Fusarium* and *Rhizopus* spp

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Introduction: In recent years *Rhizopus* and *Fusarium* had been the main cause of postharvest decay as well as major economic losses and food spoilage in various horticultural products. For years, fungal chemical control has been used; however, these products represent a potential hazard to the environment and the human being. An alternative is searching for natural antimicrobial compounds, such as thymol and carvacrol which could be used in fungal control.

Purpose: The aim of this study was to evaluate the *in vitro* antifungal activity of thymol and carvacrol mixtures against *Rhizopus* and *Fusarium* species.

Methods: Mold radial growth was evaluated to determined minimal inhibitory concentration (MIC) with thymol or carvacrol concentrations of 0 to 1600 ppm. Binary mixtures were evaluated using concentrations below the MIC values. In order to evaluate synergism or antagonism effects. Radial growth curves were described by the Gompertz modified equation.

Results: MIC carvacrol values were 200 ppm for both molds. Meanwhile, MIC thymol values ranged between 500 and 400 ppm for *Rhizopus* spp. and *Fusarium* spp., respectively. A synergistic effect below MIC concentrations for carvacrol (50 ppm) and thymol (300-375 ppm) was observed. Significant differences ($P < 0.05$) between the Gompertz parameters for the antimicrobial concentrations and their tested binary mixtures established an inverse relationship between antimicrobial concentration and mycelial development of both molds.

Significance: Thymol and carvacrol possess a synergistic antifungal activity on *Rhizopus* spp. and *Fusarium* spp. when applied as binary mixtures.

P2-78 Effect of Alginate Coatings Containing Essential Oils and Alcoholic Beverages on Growth of *Listeria monocytogenes* in Modified Atmosphere Packaged Apples, Pears and Bananas

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Developing Scientist Competitor

Introduction: Consumption of fresh cut fruits has been associated with foodborne outbreaks; therefore, the application of natural alternatives e.g., essential oils and alcoholic beverages may reduce the risk of pathogens growth and survival on these products.

Purpose: To evaluate the effect of sodium alginate coatings containing cinnamon and mastic gum essential oils (EOs)—alone or in mixtures—and their flavored liqueurs, on inhibition of *Listeria monocytogenes* growth on cut fruits under modified atmosphere packaging (MAP).

Methods: Fresh cut apples, pears and bananas were inoculated (10^3-10^4 CFU/g) with 3-strain *L. monocytogenes* mixed culture. Fruits were coated with 1% w/v sodium alginate (EC) with 0.7% (v/v) cinnamon EO (ECC); 0.7% (v/v) mastic gum EO (ECM), 0.7% (v/v) ECC+M (1:1); mastic gum liqueur (2 EC:1 liqueur) (ECML); cinnamon liqueur (2:1) (ECCL), and 25% ethanol (2 EC: 1 Ethanol) (ECE) (both liqueurs had 25% (v/v) alcohol) or left uncoated C. Samples were packaged under MAP (80%N_2: 20% CO_2) and stored at 10 °C. Enumeration of *L. monocytogenes* and total viable counts (TVC) were carried out on PALCAM and TSAYE, respectively, (n=4). PH, gas composition of package headspace and sensory properties (odor, appearance) of fruits were recorded.

Results: Maximum growth populations of *L. monocytogenes* were obtained at 13th storage day for controls (7.1 log CFU/g on apples and 8.5 CFU/g on pears and bananas). ECM had no significant inhibitory effect on pathogen growth, nor in TVC, ECCL maintained the levels of *L. monocytogenes* on apple 2.5 log CFU/g lower than the respective control on day 13, while ECC and ECE samples, remained 2 log CFU/g lower than the control on pears. ECC, ECML and ECE were the most effective for banana causing (all three of them) an inhibition of approx. 1.5 log CFU/g compared to control). PH values slightly increased in most samples (*ca* up to 0.8 pH units) until 13th day. No significant changes in O_2 or CO_2 content were observed.

Significance: The combined use of antimicrobial edible coatings and MAP could be a natural preservation method to control growth of *L. monocytogenes* on fresh cut fruits.

P2-79 Survival and Growth of *Escherichia coli* O157:H7 in the Presence of Caffeine and Cinnamon

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Introduction: *Escherichia coli* O157:H7 has emerged with increasing frequency as a foodborne pathogen of concern, which causes hemorrhagic colitis and hemolytic uremic syndrome. Caffeine and cinnamon have shown to exert strong antimicrobial effects against *E. coli* O157:H7. This suggests the potential use of caffeine in combination with cinnamon in the food industry for enhancement of safety.

Purpose: The objective of this study was to investigate the effect of caffeine and cinnamon against *E. coli* O157:H7 in laboratory medium.

Methods: Tryptic Soy Broth (TSB) was prepared with caffeine (Ca 0, 0.3, 0.4, and 0.5%, w/v), cinnamon (Ci 0.3 and 0.5%, w/v) and combination of Ca 0.4 %, w/v and Ci 0.5%, w/v. Three strains of *E. coli* O157:H7 were individually inoculated (approximately 3.00 log CFU/ml) into each sample and incubated at 37 °C for 48 hr. At the end of the incubation period, samples were analyzed for bacterial population.

Results: At 0.3, 0.4%, and 0.5%, Ca decreased the bacterial population by 1.54, 3.0, and 4.0 log CFU/ml, respectively, while Ci 0.3 and 0.5% decreased by only 0.72 and 1.07 log CFU/ml, respectively. The population of *E. coli* O157:H7 was, however, decreased by 4.0 log CFU/ml with the

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combination of caffeine 0.4% and cinnamon 0.5 %.

Significance: Our results indicate that caffeine and cinnamon are effective antimicrobials to control the survival and growth *E. coli* O157:H7. This could potentially be used as a better alternative to artificial preservatives in controlling foodborne pathogens in different liquid foods.

P2-80 Treatment with Warm Water Containing Ethanol for Controlling *Salmonella* spp. on Post-harvest Mangos

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Introduction: An outbreak of salmonellosis in the United States associated with consumption of Brazilian mangos has been documented. A hot water immersion treatment to kill fly larvae is thought to be responsible for contamination. Alternative treatments such as a mixture of warm water and ethanol have not been evaluated for effectiveness in killing *Salmonella* on mangos.

Purpose: To evaluate the combined effects of warm water and ethanol to control *Salmonella* on mangos.

Methods: Mangos were spot-inoculated with *Salmonella*, dried, and immersed in water containing ethanol (0, 1, 3, 7, and 9%) at 46°C for 70 min, then cooled in water at 21°C for 30 min. Populations of *Salmonella* on mangos were evaluated before and after treatments. Physical-chemical analysis of treated and control mangos stored for up to 7 days at 25°C and 75% RH were also performed.

Results: An initial population of *Salmonella* on inoculated mangos (5.7 log CFU/g) was reduced to an undetectable level (less than 1.0 log CFU/g) on mangoes treated with warm water containing 3 – 9% ethanol. Reduction on mangos treated with warm water containing no ethanol or 1% ethanol were 3.0 and 3.8 log CFU/g, respectively. Treatment did not affect storage quality.

Significance: Treatment of mangos with warm (46°C) water containing ethanol (3%) for 70 min is effective in reducing *Salmonella* by at least 4.7 log CFU/g without compromising quality.

P2-81 Extruded Antimicrobial Film Targeting Gram-positive Pathogens

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Developing Scientist Competitor

Introduction: Antimicrobial food packaging can help reduce spoilage, maintain food quality, extend shelf life and reduce or eliminate foodborne pathogens in ready-to-eat deli meat. Nisin is a polypeptide with natural antimicrobial activity against Gram-positive pathogens, including *Listeria monocytogenes*. As a food additive, it is generally recognized as safe (GRAS) in the United States.

Purpose: The goal of the study is to make a nisin-containing film that demonstrates antimicrobial activity.

Methods: Carver heat pressed studies using low density polyethylene (LDPE) and linear low density polyethylene (LLDPE) were performed at different temperatures (130 °C, 140 °C, 150 °C and 160 °C) and pressed five times each to incorporate 5,000 IU/g of nisin A, nisin Z, or nisin (Sigma). The heat pressed films were tested for inhibition against *L. monocytogenes* using the plate overlay assay and inhibition zones were measured in millimeters (mm) from the edge of the film. Also, LLDPE were cast extruded at 130 °C and 170 °C and with 5,000 IU/g of nisin Z or sigma nisin and then heat pressed into a film. This film was tested for antimicrobial activity against *L. monocytogenes* using a plate overlay method.

Results: All temperatures tested for the carver press study showed inhibition against Gram-positive bacteria with nisin Z demonstrating the largest inhibition zones. Inhibition for all nisin subtypes measured from 1 mm to 16 mm against all temperatures tested in the carver press. For extrusion studies, inhibition measured from 3.6 mm to 7.1 mm in LLDPE against *L. monocytogenes*.

Significance: This research is essential to the development of an antimicrobial vacuum skin package (VSP) for ready-to-eat (RTE) meat, which inhibits Gram-positive pathogens.

P2-82 Isolation and Characterization of Bacteriophage for the Control of Enterohemorrhagic *Escherichia coli* on Fresh Produce

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Introduction: Bacteriophage is a potentially applicable agent for the control of foodborne pathogens. It is particularly relevant to the safety of fresh produce and other minimally processed products since application of phage is a non-destructive treatment.

Purpose: The purpose of this study was to obtain bacteriophages specific for pathogenic *Escherichia coli* from environmental sources and evaluate their ability to control the host pathogen on fresh produce.

Methods: A phage active against *E. coli* O157:H7 EDL933, with a titer of 10^8 PFU/ml, was isolated from waste water. Cross-reactivity studies showed this phage had strong activity against eight of twelve additional *E. coli* strains. Application of phage for biocontrol was evaluated on green peppers. Cut peppers were treated with UV light to eliminate background biota, then spot-inoculated with *E. coli* O157:H7 EDL933 on cut edges. Inoculated pepper was dipped for 2 min in phage lysate, stored at room temperature, and plated periodically for up to 72 h. Control samples were dipped for 2 min in LB broth, the same medium used in the lysate, and analyzed at the same time intervals.

Results: Phage treatment inactivated 1 log *E. coli*/g pepper, compared to the initial load following rinsing, and 2 logs compared to the control population after 3 h. After 72 h this difference between the treatment and the control was reduced to 1 log due to recovery of surviving cells. Reductions could be increased by modifying experimental parameters such as dip time and titer.

Significance: These findings suggest the usability of bacteriophage to selectively control pathogens on fresh produce without damaging the product. Further testing is warranted to evaluate phage biocontrol in other food products as well.

P2-83 Synergistic Effects of Clove and Lemon Essential Oils against *Listeria monocytogenes* Strains

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Introduction: Little is known about the synergistic actions between fractions of essential oils used in the food industry as natural agents for food preservation.

Purpose: To evaluate the synergistic effects of clove (C) and lemon (L) essential oils by determining the minimum inhibitory concentration against *Listeria monocytogenes* strains.

Methods: The minimum inhibitory concentration (MIC) was determined by using the broth dilution method for testing six isolates of *L. monocytogenes* (ATCC 35152 and five isolates from meat products). Each culture (10^6 CFU/mL) was inoculated in 5 mL tryptic soy broth plus yeast

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extract (TSB-YE) tubes containing different concentrations of the essential oils. After 24h at 37 ± 1° C, 100 µL of each tube was spread plated on the surface of trypticase soy agar plus-YE and incubated for 37 ± 1° C for 24 h. Positive and negative controls were used. After determining the MIC, the essential oil fractions were mixed at different proportions (100, 75/25, 50/50, 50/25, 25/25%, v/v) in which 100% was the MIC obtained for each oil. The presence or absence of colonies using the broth dilution method was tested.

Results: The *L. monocytogenes* ATCC 35152 was the most sensitive against both essential oils evaluated with MIC values of 0.05 and 0.2% for clove and lemon essential oils, respectively. For all other strains, the MIC value was higher - 0.1 and 0.3% - respectively, for those essential oils. The inhibitory effect was also achieved by using a combination of 75/25 (C/L), 50/50 (C/L), 50/25 (C/L) and 75/25 (L/C).

Significance: The results showed the synergistic effects between fractions of clove and lemon essential oils tested against *L. monocytogenes* strains. The combination of essential oils can reduce the concentrations used and therefore minimizing sensorial impact in food.

P2-84 Efficacy of a Novel Proteinaceous Antifungal Agent in Fruit Juices and Teas

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Introduction: Heat-resistant fungal spores present a challenge in the processing of many types of beverages and fruit juices. Thermal and ultraviolet processing have limited effectiveness for reducing heat resistant fungal burdens to levels that will ensure both a safe and stable product.

Purpose: We illustrate the utilization of a novel, recently identified antifungal protein derived from *Bacillus thuringiensis* as a potential protectant against fungal spoilage.

Methods: Juice and tea matrices were inoculated with approximately 5-log *Byssochlamys fulva* H25 spores and incubated with serial two-fold dilutions of semi-purified antifungal protein. Minimum inhibitory concentrations (MICs) were evaluated in triplicate using a modification of the XTT cell proliferation assay while ambient temperature shelf life studies were inspected daily for visual signs of mold growth.

Results: The antifungal was active over a wide range of fruit juices and acidified tea beverages with MICs ranging from 1.56 ppm in apple juice and select acidified teas to approximately 3-12 ppm for lemonade, orange, white cranberry, blueberry, prune, cherry and grape juices. Apple cider and non-acidified teas generally exhibit a reduced efficacy with MICs exceeding 100 ppm. The antifungal extended the shelf life of each product in a concentration-dependent manner. Shelf lives ranged from a few days to several weeks depending on the concentration of protein utilized. However, initial efficacy did not seem to be a predictive indicator of shelf life. Lemonade, white cranberry and apple juice showed a high level of protection at just two and four fold concentrations above the MIC while apple cider, acidified black tea and blueberry juice only exhibited long-term stability at antifungal concentrations exceeding 20 times the MIC.

Significance: This protein is attractive as a potential alternative to currently available compounds and will provide a greater diversity of natural food protectants to combat fungal spoilage in beverages and fruit juices.

P2-85 Validation and Microbiological Performance of Antimicrobial Agents within Poultry Processing Plants

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Introduction: Poultry slaughter processes utilize antimicrobial agents in processing water for water reuse programs and to decrease microbial populations on carcasses.

Purpose: The purpose of this study was to identify antimicrobial applications and parameters that best reduce microbial populations on poultry carcasses using in-plant generated data.

Methods: In six poultry processing facilities, antimicrobial solutions were applied during inside-outside bird washing (IOBW), online reprocessing (OLR), hydro-chilling (HC), and post-chiller antimicrobial wash (PCAW) and consisted of acidified sodium chlorite (ASC), buffered sulfuric acid (BSA), chlorine (C), or peroxyacids (PA) that were applied as a topical carcass spray or whole carcass immersion using a dip tank. Before and after each process, bacteria were rinsed from carcasses (1 min, 400 ml Butterfield's phosphate solution). Rinsates (4 replicates, 7 samples/step/repetition) were neutralized via pH adjustment (6.5-8.0), and analyzed for total aerobic bacterial and *Escherichia coli* populations. Microbiological data were analyzed by plant using the general linear model of Minitab and means were separated (α=0.05) using Tukey's honestly significant differences test.

Results: Recovered *E. coli* populations were numerically fewer than total aerobic bacteria; however, general trends in data were conserved. In all plants, multiple antimicrobial applications lowered ($P < 0.05$) *E. coli* population from 1.9-2.3 to <0.0-0.7 log CFU. *E. coli* populations after OLR tended to be fewer with a dip (ASC, 15 s) application than spray (ASC, BSA; 8 s) application (0.5-0.6 and 0.7-1.4 log CFU, respectively). After HC, results were inconsistent and no clear trends were observed based on antimicrobial agent (C, PA; 1.5 h) or concentration (17-54 ppm). Undetectable levels (< 0.0 log CFU) of *E. coli* were observed after the PCAW in plants that implemented an immersion style intervention (ASC, BSA; 15 s).

Significance: These data can be used as validation for in-plant application parameters and suggest the best performance of an antimicrobial agent is in a dip-style application for at least 15 s at OLR and PCAW.

P2-86 Combined Effects of Naturally Occurring Antimicrobial Agents in Inhibiting the Growth of *Bacillus cereus* in Infant Rice Cereal

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Developing Scientist Competitor

Introduction: One of the most frequently used methods to prevent the growth of foodborne pathogens in food is the addition of chemical agents as a preservative. However, consumer acceptance for the addition of synthetic chemicals is low due to their potential toxicity. Therefore, the addition of naturally occurring antimicrobial agents in foods to inhibit the growth of foodborne pathogens has been interested.

Purpose: The objectives of this study were to screen natural antimicrobial agents against *Bacillus cereus*, to test synergistic lethal activities between natural agents, and to confirm their lethal activity against *B. cereus* in infant rice cereal.

Methods: Plant extracts (2,117 types) were tested for their antimicrobial activity against *B. cereus* using well diffusion assay. For selected 13 plant extracts, their minimal inhibitory concentration (MIC) against *B. cereus* was determined. Combined effects between different extracts against *B. cereus* were evaluated using a checkerboard test. Finally, the lethal activity of combined extracts against *B. cereus* was tested in infant rice cereal.

Results: *Siegesbeckia glabrescens* and *Morus alba* extracts showed the lowest MICs (0.03 mg/ml) followed by *Dryopteris erythrosora* (0.06 mg/ml) and *Carex pumila* (0.06 mg/ml) in tryptic soy broth. A combination of *S. glabrescens* and *C. pumila* and a combination

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of *D. erythrosora* and *C. pumila* showed partial synergistic effects against *B. cereus* with the fractional inhibitory concentration index (FICI) of 0.63. MICs of *S. glabrescens, M. alba, D. erythrosora*, and *C. pumila* extracts were significantly increased to 1, 2, 2, and 8 mg/ml, respectively, in infant rice cereal. The combination of *D. erythrosora* (1 mg/ml) and *C. pumila* (1 mg/ml) extracts showed partial synergistic lethal activity (FICI = 0.63) in infant cereal.

Significance: Natural antimicrobial agents to inhibit *B. cereus* were screened. Their individual or combined antimicrobial activities were characterized using a laboratory medium and infant cereal.

P2-87 Antibacterial Activities of *Clitocybe nuda* Mushroom Extract on Foodborne Pathogens

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Introduction: Foodborne pathogens cause serious food safety issues every year. This situation has forced scientists to search for novel antimicrobial agents. *Clitocybe nuda* mushroom is an edible macrofungi which produces biologically active compounds with antibacterial activities.

Purpose: The purpose of this study was to evaluate the antibacterial activities of *Clitocybe nuda* extract on foodborne pathogens and its stability at different temperatures and pHs.

Methods: *Clitocybe nuda* mushroom was dried, ground, extracted in 95% ethanol and finally redissolved in deionized water. The antimicrobial activities were evaluated by testing the minimum inhibitory concentration (MIC) on *Listeria monocytogenes*, *Salmonella* Typhimurium, *E. coli* O157:H7, and *Staphylococcus aureus*. The extract was treated at 4, 25, 72, 100, and 121 °C for 15 min and at pH 4-10 for stability test. **Results** were calculated and compared using ANOVA.

Results: The mushroom extract showed strong antimicrobial activities on the tested foodborne bacteria. The MIC50 were 77.04 ± 3.05 mg/ml for *Listeria monocytogenes* and 77.05 ± 1.73 mg/ml for *Salmonella* Typhimurium. The extract is heat stable and the antibacterial activities between different temperature treated samples were not significantly different ($P \geq 0.05$). The change of pH had no negative effect on the antibacterial activities of this extract.

Significance: The results indicated that the extract can effectively control the growth of foodborne pathogens. There is a great potential use in foods due to its stability at high temperature and in a wide range of pH.

P2-88 Inhibitory Effect of Xoconostle (*Opuntia matudae*) on the Growth of *Salmonella* and *Escherichia coli* O157:H7

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Introduction: Antimicrobial agents, including natural and chemical preservatives, have been used to inhibit foodborne pathogens and extend the shelf life of processed food. Plants, herbs, and spices are rich in naturally occurring compounds that have many health benefits and could have strong inhibitory effect on the growth of foodborne pathogens. Xoconostle (*Opuntia matudae*) has demonstrated strong anticancer and antioxidant characteristics and may contribute to inhibitory effect.

Purpose: This study focuses the inhibitory effect of xoconostle pears against the growth of *Salmonella* and *Escherichia coli* O157:H7 in culture medium BHI.

Methods: Seven strains of *Salmonella* and four strains of *E. coli* O157:H7 were individually tested with xoconostle extract. Growth over time assay and agar well diffusion assay were used to determine the inhibitory effect of xoconostle extract.

Results: Results showed that bacterial population in control for *Salmonella* and *E. coli* O157:H7 reached an average of 9.73 ± 0.153 and 8.96 ± 0.315 logs CFU/ml respectively, while the addition of 10% xoconostle extract caused the bacterial population to remain within 2.82 ± 0.115 and 3.48 ± 0.317 logs CFU/ml respectively. The minimum inhibitory volumes (MIV), the lowest concentration that has significant inhibitory effect after 10 – 12 h of incubation at 37 °C, for*Salmonella* and *E. coli* O157:H7 were 200 and 275 µl/ml (V/V) respectively and the minimum lethal inhibitory volumes (MLV), the lowest concentration that has significant inhibitory effect after three days of incubation at 37 °C, for *Salmonella* and *E. coli*were 450 and 550 µl/ml (V/V), respectively.

Significance: These results indicated that xoconostle has potential inhibitory effect against the growth *Salmonella* and *E. coli*. Xoconostle could provide a natural means of controlling pathogenic contamination, thereby mitigating food safety risks.

P2-89 Identification of a Bacteriocinogenic Lactic Acid Bacteria Isolated from Raw Cow's Milk and Partial Characterization of its Antilisterial Bacteriocin

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Introduction: There is a demand for safe, healthy and minimally processed foods, and this stimulates the search for new antimicrobial agents, such as the bacteriocins of lactic acid bacteria. Bacteriocins are peptides ribosomally synthesized by bacteria and can be degraded by digestive enzymes, representing a potentially safe alternative to classical food preservatives.

Purpose: To study a milk isolate bacteriocin-producing lactic acid bacterium strain (LAB 126) by phenotypical and molecular identification and partial physico-chemical characterization of the antimicrobial peptide produced.

Methods: LAB 126 was isolated in Brazil from raw cow's milk and it was identified at genus level by Gram stain, catalase test, gas production from glucose, growth in regular MRS broth at 10 and 45 °C, and in 2, 4 and 6.5% NaCl MRS broth. Molecular identification by sequencing of 16S rDNA is under progress. Antagonistic activity was tested against *Listeria monocytogenes* and other food related bacteria, using the spot-on-the-lawn agar assay. A modified well-diffusion agar assay was used to evaluate the sensitivity of the inhibitor to proteases (type XIV from *Streptomyces griseus*, proteinase K and chymotrypsin). To exclude bacterial inhibition due to acid production, filter-sterilized neutralized supernatant from overnight LAB 126 MRS broth culture was spotted (10µl) on BHI agar plate, previously overlaid with soft BHI agar seeded with the indicator strain *L. monocytogenes* (ca. 10^5 CFU/ml). The bacteriocin produced by LAB 126 was purified from a neutralized cell-free supernatant of a BHI broth culture grown at 25 °C/24 h (crude extract) using Amberlite® XAD-16 resin (Sigma-Aldrich, EUA), followed by solid phase extraction – SPE (HF Mega Bond Elut C18 10 g-60 ml, Varian, EUA). Extracts containing bacteriocin were analysed by tricine SDS-PAGE and antimicrobial activity in extracts was measured by critical dilution assay using *L. monocytogenes* as indicator strain and expressed as arbitrary units per ml (AU/ml).

Results: LAB 126 was identified as *Lactococcus* sp. by phenotypical tests and produced an inhibitory compound susceptible to degradation by proteases, indicating it was a bacteriocin. The bacteriocin was active against *L. monocytogenes*,*Listeria innocua* and other lactic acid bacteria tested.

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Crude extract from BHI culture of *Lactococcus* sp. 126 presented an activity of 6,400 AU/ml against *L. monocytogenes*. The yield of the partial purification carried out with XAD-SPE was calculated as 27%, by comparing total activities from crude and final extracts. Results from SDS-PAGE assays showed the molecular weight of the bacteriocin is in the range of 3.5 to 8.5 kDa.

Significance: The isolation of bacteriocin-producing LAB and the characterization of novel bacteriocins can contribute for improving food safety by providing new alternatives for food biopreservation approaches.

P2-90 Effect of Different Coating Treatments on Population of *Salmonella* spp. and Quality of Cherry Tomatoes

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Introduction: Coatings can extend the shelf life of fruits and vegetables by functioning as gas and vapor barriers. Incorporation of antimicrobial compounds such as essential oils into coatings may reduce pathogens in addition to prolonging shelf life.

Purpose: This study was conducted to investigate the effectiveness of zein and chitosan-acids based coatings in reducing population of *Salmonella* spp. on tomatoes while preserving fruit quality.

Methods: Zein-based coatings with 0-20% cinnamon and mustard essential oil and chitosan-acids coatings with 60 ppm mustard essential oil were developed and applied to cherry tomatoes. Populations of *Salmonella* spp. on the fruit were enumerated during post-coating storage. Additionally, quality of tomatoes was evaluated.

Results: Our results showed that population of attenuated *S.* Typhimurium inoculated on the smooth surface and stem area of the tomatoes were reduced with the increasing essential oil concentration and storage times. *S.* Typhimurium population on the smooth surface of tomatoes was reduced by 4.59 and 2.84 log CFU/g by the zein coatings with 20% cinnamon and 20% mustard oil, respectively, 5 hours after coating. The coatings reduced population of *S.* Typhimurium by 1.47-6.31 log CFU/g on the stem area of the fruit during 7 days storage at 10 °C. The chitosan-acids coatings reduced more than 3.56 log CFU of *S.* Stanley, *S.* Panama and *S.* Poona on the whole tomatoes. The loss of firmness during storage was retarded by all of the coatings except the zein coating with 20% mustard oil which induced fruit softening. Color, soluble solids content, pH, titratable acidity and ascorbic acid of tomatoes were not consistently affected by any of the coating treatments.

Significance: Our results suggest that the zein or chitosan-based coatings containing essential oils may be used to enhance microbial safety and quality of tomatoes.

P2-91 Inhibition Activity of Lactic Acid Bacteria against *Salmonella*, *Escherichia coli* O157:H7 and Non-O157 STECs in Ground Beef

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Introduction: *Salmonella, Escherichia coli* O157:H7 and non-O157 STEC are pathogens capable of causing high morbidity and mortality among humans, with the majority of infections linked to the consumption of contaminated meat and meat products. Although many intervention methods are commonly applied to beef carcasses, very few interventions have been validated to be effective in ground beef.

Purpose: The objective of this study was to evaluate the effect of different cocktails of lactic acid bacteria (LAB) on the growth and survival of *Salmonella*, *Escherichia coli* O157:H7 and non-O157 STECs in ground beef during refrigerated storage.

Methods: Four LAB cocktails including NP51+C28, NP51+C28+D3, NP51+C28+L7+D3 and NP51+D3+L7+M35 were added at 1 x 10^7 CFU/g of ground beef containing a pathogen concentration of 10^3 CFU/g. Samples were stored at 4 °C , and pathogen enumeration was evaluated on days 0, 1, 3 and 5.

Results: Compared with control sample, there were significant ($P < 0.05$) reductions on all of the pathogens in the ground beef samples treated with all LAB cocktails during 5 days of refrigerated storage. LAB cocktails of NP51+D3+L7+M35 and NP51+C28+D3 displayed significantly stronger ($P < 0.05$) inhibition activities than the other two (NP51+C28 and NP51+D3+L7+M35). On day 5, the most significant reductions on *Salmonella* (1.42 log CFU/g), *Escherichia coli* O157:H7 (1.45 log CFU/g) and non-O157 STEC (1.08 log CFU/g) were observed after the application of NP51+D3+L7+M35.

Significance: The treatment of LAB cocktails is a very effective intervention for ground beef to control *Salmonella*, *Escherichia coli* O157:H7 and non-O157 STECs. LAB cocktails can be provided to processors in various forms (frozen, liquid or freeze-dried) and application can be easily implemented into current operations by adding the cocktails into ground beef products during processing.

P2-92 Biopreservation: Control of *Listeria monocytogenes* Growth in Hot and Cold Smoked Salmon by *Carnobacterium maltaromaticum* CB1

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Introduction: Recent outbreaks of *Listeria monocytogenes* in Canada and the US have captured significant attention by media and public to the risks of consuming minimally processed ready-to-eat foods. The risks associated with the consumption of *L. monocytogenes* in post-process contaminated smoked salmon are increased by their intrinsic properties, high water activity, pH, and salt, and minimal smoking, favoring the growth of *L. monocytogenes*. Health authorities require that manufacturers must validate no more than 0.5 log growth of *L. monocytogenes* over the product's shelf-life.

Purpose: To determine if the application of a live bioprotective bacterium *Carnobacterium maltaromaticum* CB1 inhibits the growth of *L. monocytogenes* when applied to the surface of hot or cold smoked salmon.

Methods: Hot and cold smoked salmon was obtained from a commercial manufacturer and shipped on ice to the testing facility. A four-strain cocktail of biocompatible *L. monocytogenes* was used to inoculate the salmon surface at approximately 10^3 CFU/g. The product was additionally inoculated with the bioprotective culture *C. maltaromaticum* CB1 at 10^4 CFU/g. Product was vacuum packaged and stored at 4 °C for 28 days. Samples were enumerated every 3 or 4 days for total plate counts, *L. monocytogenes,* and *Carnobacterium* spp.*,* by plating onto the surface of Plate Count, PALCAM, and Cresol red Thallium acetate Sucrose Inulin agars, respectively.

Results: *C. maltaromaticum* CB1 inhibited the growth of the cocktail of *L. monocytogenes* on hot and on cold smoked salmon for at least 7 days compared to control product. *C. maltaromaticum* CB1 grew well on the hot smoked salmon, but minimally on the cold smoked product. The slow growth of *C. maltaromaticum* CB1on the cold smoked salmon did not suppress its ability to control the growth of the cocktail of *L. monocytogenes*.

Significance: The bioprotective culture *C. maltaromaticum* can extend the commercial shelf life of both hot and cold smoked salmon when growth of *L. monocytogenes* by 0.5 logs is used as the measure of shelf life.

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P2-93 Antimicrobial Properties and Mutagenicity of Sappanwood *(Caesalpinia sappan L.)* Water Extract

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Introduction: Red coloring extracted from sappan heartwood has been used as a colorant in beverages in Thailand and Indonesia. These extracts may also have potential as food preservative agents, as they have been shown to have both antioxidative (in beef patties and peanut oils) and antimicrobial properties (in chili paste).

Purpose: We evaluated the extract's antimicrobial properties against spoilage bacteria (*Alcaligenes faecali*, *Bacillus coagulans,* and *Pseudomonas putida)* as well as its potential toxicity (using the Ames test) in order to provide additional information on functional properties and safety.

Methods: We tested freeze-dried samples of extracts of sappan heartwood extracted in hot water (5 hours reflux (HW)) and cold water (6 hours of shaking at room temperature (CW)). Antimicrobial tests were conducted using the disc diffusion method, while mutagenicity tests were accomplished using the Ames mutagenicity assay.

Results: Freeze-dried HW and CW extracts both exhibited antimicrobial activity against the tested bacterial strains; there were no significant differences ($P > 0.5$) between HW and CW samples in terms of their antimicrobial activity. *B. coagulans* was the most sensitive to the extracts, with an inhibition zone of about 18-19 mm at applications of 150 µg of dried extract per disc. At 1.5 mg per disc, the extracts effectively inhibited the growth of *A. faecalis* (25 mm) but not *P. putida* (9 mm). An Ames assay performed on *Salmonella* Typhimuriam TA 98 and TA 100 showed that neither HW extracts nor CW extracts exhibited mutagenicity at concentrations as high as 5 mg per plate.

Significance: These results demonstrate that sappanwood extract may have potential as a safe and natural food preservative.

P2-94 Antimicrobial Drug Resistance Patterns among Cattle and Human Associated *Salmonella*: Are Cattle a Reservoir for Multidrug-resistant *Salmonella* Linked to Human Disease?

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Introduction: *Salmonella,* an important human and animal pathogen worldwide, is transmitted to humans through contaminated food, water or through direct contact with infected hosts. The emergence and spread of multidrug-resistant strains of *Salmonella* has become a public health concern worldwide. Antimicrobial susceptibility is commonly measured by either broth or agar dilution methods or agar diffusion techniques. Phenotypic methods for determining antimicrobial resistance profiles of *Salmonella* isolates are widely used for surveillance and diagnostic applications. However, numerous drawbacks can potentially limit the usefulness of such approaches. In house genotypic screening for the absence or presence of genes responsible of antimicrobial resistance may potentially offer an alternative to phenotypic screening, and may provide rapid, reliable, and cost-effective antimicrobial sensitivity results.

Purpose: Our purpose in this study is to better understand the transmission dynamics and genetic determinants of antimicrobial resistance of *Salmonella* isolates among human and bovine hosts using phenotypic and genotypic methods.

Methods: Here we described the distribution of multidrug resistance among 336 *Salmonella* isolates from human and cattle, representing 51 different serotypes. We also investigated genetic determinants of antimicrobial resistance among both human and bovine isolates, which have previously been characterized by MLST and PFGE. Here we tested all isolates for susceptibility to 15 antimicrobial drugs that are part of the National Antimicrobial Monitoring System (NARMS) bovine susceptibility panel. PCR was performed on a representative subset of unique isolates (n=53) to screen for the presence of 21 known antimicrobial resistance genes; selected fluoroquinolone and nalidixic acid-resistant (n=3) and sensitive (n=6) isolates were also tested for known resistance conferring mutations in *gyrA* and *parC*.

Results: Overall, our data confirm that bovine *Salmonella* isolates have a significantly higher incidence of multidrug resistance (MDR) than human *Salmonella* isolates. Importantly, genes responsible for antimicirobal resistance are shared among isolates of human and bovine origin, and diverse genotypes can result in similar phenotypic resistance profiles. Notably, the presence of *flo, aadA1*, and *aphA1-iab* genes and groups of *sul* genes (*sulI* and *sulII*) and group of *tet* genes (*tetA, tetB*and *tetG*) indicates phenotypic profile of Chloramphenicol, Gentamicin, Kanamycin, Sulfizoxazole and Tetracycline, respectively, in our *Salmonella* isolate set, regardless of source of isolates.

Significance: Our study characterizes the molecular determinants of antimicrobial resistance among *Salmonella* isolates from humans and cattle, and our findings are consistent with a role of cattle as one important source of human MDR*Salmonella* strains. In addition, we conclude that instead of one gene, a set of genes should be used to determine antimicrobial profiles of *Salmonella* isolates.

P2-95 Prevalence of Lactose Fermenting Coliforms Resistant to Third Generation Cephalosporins in a Cattle Feedlot Throughout a Production Cycle and Molecular Characterization of Resistant Isolates

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Introduction: Increases in incidence of human infections caused by Enterobacteriaceae resistant to 3rd-generation cephalosporins (3GC) have become a public health concern. The 3GC ceftiofur is commonly used for the therapeutic treatment of feedlot cattle but the impact this practice has on public health is not clear.

Purpose: To determine the prevalence of lactose-fermenting coliforms resistant to the 3GC cefotaxime (Lac+ CTX) in feces and on hides of feedlot cattle throughout a production cycle and molecularly characterize the resistant isolates.

Methods: Fecal samples (n = 1,446) and hide samples (n = 1,446) were taken on six occasions over the 10-month period which the study population of cattle (n = 763) resided at the feedlot. Samples were plated onto MacConkey agar supplemented with 4 mg/l cefotaxime to isolate Lac+ CTX. Prevalences were evaluated by chi-square with Bonferroni's correction for multiple comparisons and comparisons with P values < 0.01 were considered significant. Molecular characterization included plasmid Inc group identification, plasmid size analysis, PCR for presence of *blaCMY-2* gene, and pulsed field gel electrophoresis (PFGE) sub-typing.

Results: The fecal Lac+ CTX prevalence of 27.8% during the period of frequent ceftiofur use was higher than the 4.3% prevalence when cattle arrived at the feedlot and the 1.1% to 9.4% prevalences during periods of infrequent ceftiofur use at the feedlot. The 15.5% hide Lac+ CTX prevalence when cattle arrived at the feedlot was not different from the 13.9% prevalence during the period of frequent ceftiofur use and the 1.9 to 19.3% prevalences during periods of infrequent use. Molecular analysis of 383 resistant isolates revealed that the resistance plasmid conferring 3GC resistance, as well as resistance to other antibiotic classes, integrated into the chromosome of the most frequently isolated Lac+ CTX sub-type.

Significance: Lac+ CTX proportion of the total Lac+ coliform feedlot population increases during periods of frequent ceftiofur use but returns to baseline levels when ceftiofur is not used. Molecular analysis of resistant isolates revealed that the plasmid conferring resistance to 3GC

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integrates into the chromosome, which may contribute to maintenance of 3GC resistance in the absence of selective pressure (ceftiofur use).

P2-96 Detection of Carbapenemase-producing *Enterobacteriaceae* from Dried Indian Spices

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Introduction: Enterobacteriaceae with the emerging New Delhi metallo-lactamase-1 (NDM-1) (carbapenemase) provides bacteria with antibiotic resistance to all -lactam antibiotics except aztreonam, leading to great concern in public health globally.

Purpose: The objectives of this study were to determine if the imported spices from India could serve as a vector for the spread of NDM-1-positive Enterobacteriaceae.

Methods: Samples (n=28) of the imported dried spices produced in India were collected from an international market located in Atlanta, GA. Each spice sample was weighed and analyzed microbiologically. Serial dilutions of each spice were then plated on TSA, ESBL, KPC, and coliform petrifilm plates. Both ESBL and KPC plates detected the presence of the NDM-1-producing bacteria. The selected NDM-1-producing colonies were picked, purified, tested for susceptibility to 15 antibiotics by determining minimum inhibition concentration (MIC), and further tested for species level and the gene encoding NDM-1.

Results: Total bacterial counts on TSA ranged from <100 to 7.3×10^6 CFU/g with the highest count in the black pepper sample. Ten samples were positive for coliforms ranging from <5 to 2.5×10^4 CFU/g, with the highest count in the ground turmeric. Seven samples (Garam Masala, turmeric powder, mustard seed, fennel seed, white chili powder, ground turmeric seed, and ground coriander) were positive for NDM-1 positive bacteria with counts ranging from 750 to 1.4×10^4CFU/g and 75 to 1.1×10^4 CFU/g on ESBL and KPC, respectively. The Garam Masala sample contained the highest count on both ESBL and KPC. Among five NDM-1 positive isolates, 2 isolates were resistant to two antibiotics, whereas each of the 3 other isolates was resistant to 4, 5, and 7 antibiotics, respectively. The prevalence of antibiotic resistance was in the following order: cefoxitin, nalidixic acid, ampicillin, and ceftiofur.

Significance: Our results demonstrate that NDM-1-positive bacteria are present in imported dried spices from India, and these products could serve as a vector for the spread of the NDM-1-producing Enterobacteriaceae.

P2-97 Microscopy Study on the Effect of Essential Oils on Growth and Germination of *Aspergillus* sps in Peanuts

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Introduction: *Aspergillus flavus* infect peanuts and are known to produce carcinogenic aflatoxins. At present, pre and post-harvest afflation management in peanuts is practiced solely through adaptation of suitable agricultural practices such as crop rotation, use of good quality seed and fungicides, and altering the time of planting with adequate irrigation to avoid drought stress. According to the USDA more than $1 billion is spent on infection prevention, which contributes to poor seed quality, low yield, and price reduction and this cost is estimated to exceed $25 million per year.

Purpose: There is no direct action farmers can take to control afflation producing fungi in peanuts. Fungicides have proven to be detrimental to the environment. Increasing interest in antimicrobial agents are being studied as biological control agents against mold in grain, cereals and edible nuts. Hence, the present study investigates the synergistic effect of essential oils against *A. flavus* in peanuts.

Methods: Antifungal activity of the essential oils was tested on colony growth and spore germination using modified poisoned food method (Viuda-Martos et al., 2006). Potato dextrose agar (PDA) plates were diffused with combination of two oils and incubated for 7-10 days at 25 °C. Following incubation, minimum inhibition concentration (MIC) was determined (Hadaceck and Greger, 2000). Changes in conidial morphology and spore germination were observed by light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM).

Results: Incubation time and concentration of oils affected colony growth and spore germination. Colony diameter decreased with increase in concentration and average germ tubes were shorter (2.2 cm) when exposed to 72 h than 48 h (4.4 cm). A combination of 100 ppm of clove and 500 ppm of cinnamon, respectively, were found to be fungicidal. The lethal doses 100 and 1000 ppm inhibited about 65% of fungal growth after five days of incubation as compared to control. Disintegration of hyphal wall, disruption of plasma membrane and disorganization of cell organelles leading to large vacuoles in affected cells were observed. Scanning electron micrographs showed that while length of germ tube varied with exposure time, collapse of spores was visible at higher concentrations.

Significance: Our study revealed that essential oils affect colony growth and spore germination of *A. flavus* in peanuts. Cinnamon and clove oils showed potential for use as biological control agents. Selected oils may be studied further and integrated in control strategies and aflatoxin reduction in peanuts and other edible nuts.

P2-98 Lactic Acid Decontamination of Beef Trimmings Inoculated with *Escherichia coli* O157:H7, Non-O157 Shiga Toxin-producing *Escherichia coli*, and Multidrug Resistant and Susceptible *Salmonella* Serovars

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Introduction: Shiga toxin-producing *Escherichia coli* and *Salmonella* are of concern in meat and there is interest as to whether lactic acid decontamination treatments are adequate for their reduction in beef trimmings.

Purpose: The efficacy of lactic acid (LA) decontamination of beef trimmings was evaluated against (i) six non-O157 Shiga toxin-producing *E. coli* (nSTEC) serotypes, and (ii) antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium. The antimicrobial effects against these pathogens were compared to those obtained against *E. coli* O157:H7.

Methods: Four-strain mixture inocula of rifampicin-resistant *E. coli* O157:H7, O26, O45, O103, O111, O121 and O145, and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium were evaluated on beef trimmings (100-g pieces). The inoculated (3 log CFU/cm^2) trimmings were immersed (30 s) in solutions of LA (5%, 25 and 55 °C). Pathogen populations on untreated and treated samples were enumerated (two repetitions, three samples each), and data were analyzed as a complete randomized block design.

Results: Initial levels (3.1-3.3 log CFU/cm^2) of *E. coli* O157:H7 and nSTEC serotypes were reduced ($P < 0.05$) by 0.7 (*E. coli* O157:H7) and 0.4-0.9 (nSTEC) log CFU/cm^2 in 25 °C LA-treated samples, and 1.4 (*E. coli* O157:H7) and 1.0-1.3 (nSTEC) log CFU/cm^2 in 55 °C LA-treated samples. No differences ($P \geq 0.05$) were obtained between surviving counts of the six nSTEC serotypes and those of *E. coli* O157:H7. LA at 25 °C and 55 °C reduced ($P < 0.05$) *Salmonella* counts (3.0-3.3 log CFU/cm^2) by 1.2-1.5 and 1.5-1.9 log CFU/cm^2, respectively, while corresponding *E.*

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coli O157:H7 reductions were 0.5 and 1.2 log CFU/cm^2, respectively. Reductions of *Salmonella* counts were not influenced by serovar or antibiotic resistance profile, and were similar ($P \geq 0.05$) or higher ($P < 0.05$) than reductions of *E. coli* O157:H7.

Significance: The results indicated that LA decontamination of beef trimmings can be as effective against the six nSTEC serotypes and antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium as it is against *E. coli*O157:H7.

P2-99 Effectiveness of Sustained Antimicrobial Packaging on Control of *Escherichia coli* O157:H7 on Iceberg Lettuce

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Introduction: Leafy green vegetables contaminated with bacterial pathogens such as *E. coli* O157:H7 have been linked to several outbreaks of infections. An antimicrobial intervention that would be effective, economical, and adoptable by the fresh produce industry for control of pathogen contamination is in great demand.

Purpose: The objective of this study was to evaluate the efficacy of sustained antimicrobial packaging on control of *E. coli* O157:H7 on artificially-contaminated lettuce.

Methods: Commercial iceberg lettuce was inoculated with a three-strain mixture of *E. coli* O157:H7 at 10^1 or 10^3 CFU/g. The contaminated lettuce and un-inoculated controls were placed in each of four different antimicrobial packaging structures. Traditional, non-antimicrobial structure was included in the study as controls. The packaged lettuce was stored at 4 or 10 °C for 3 weeks. The lettuce was sampled twice a week for the population of *E. coli* O157:H7.

Results: Results showed that *E. coli* O157:H7 was not detectable from samples inoculated with 1 log *E. coli* O157:H7 and stored at 4 °C including the samples in the non-antimicrobial packaging structure. Lettuce inoculated with 3 log *E. coli* O157 and stored at 4 °C and all samples stored at 10 °C in packaging structures with the CO_2 generator, O_2 scavenger, or chlorine dioxide generator tested negative for *E. coli* O157H7 except for samples inoculated with 3 log *E. coli* O157:H7 in packaging structures with the CO_2 generator at 10 °C. The populations of *E. coli* O157:H7 in the control packaging structure had 1.85 – 5.32 log CFU/g of *E. coli* O157:H7. The packaging structure with the AIT generator was ineffective in inhibiting the growth of *E. coli* O157:H7 and the populations of the pathogen ranged from 1.30 to 5.89 log CFU/g.

Significance: The research suggests that some of the antimicrobial packaging structures evaluated in the study were effective in inhibiting the growth of *E. coli* O157:H7 on iceberg lettuce.

P2-100 Effect of Ethyl Alcohol, Propylene Glycol and Triacetin on the Survival of Vegetative Pathogens in Model Flavor Systems

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Introduction: The most commonly used carrier compounds in flavor-based systems in the food industry are ethyl alcohol, propylene glycol, or triacetin. Currently, there is limited or no literature available to support the antimicrobial properties of ethyl alcohol, propylene glycol, and triacetin in food flavor systems. This study was undertaken to evaluate the bactericidal effect of different concentrations of carrier compounds on the survival of various vegetative pathogens.

Purpose: The objectives of this study were: (1) to evaluate the antimicrobial efficacy of ethyl alcohol, propylene glycol, and triacetin on the survival of *Salmonella* spp., *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*, (2) to find the optimum concentration of each carrier compound that may be bactericidal (ex: 5-log reduction) for vegetative pathogens.

Methods: Cultures (1 ml) of *S.* Typhimurium, *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes* were added to 9 ml of nutrient broth with ethyl alcohol at concentrations of 7.5, 10.0, and 12.5 % and 15, 20, and 30% for propylene glycol to achieve an initial inoculation of 6-7 log CFU/ml. For triacetin, all the cultures were inoculated in dry form (1 g) to 9 ml of triacetin at concentrations 75, 80, and 85% to achieve an initial inoculation of 6-7 log CFU/ml. All the experiments were performed in duplicates. The samples were stored at ambient temperature. Microbiological analysis was performed following 0, 1, 3, 7, 14, 21, and 31 days of storage.

Results: Results indicated that the bactericidal effect was due to the combination of concentration and storage time. The optimum bactericidal concentrations that caused a 5-log reduction ($P < 0.05$) in vegetative pathogens were 12.5% for ethyl alcohol, 20.0 or 30.0% for propylene glycol, and 75% for triacetin.

Significance: The current study provides scientific basis for the antimicrobial effect of different chemical carrier compounds routinely used in food flavor systems.

P2-101 Flow Cytometry Analysis and Transmission Electron Microscopic Examination of *Listeria monocytogenes* Treated with Sodium Metasilicate

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Introduction: Occurrence of *Listeria monocytogenes* in ready-to-eat (RTE) food products is a significant food safety concern. Sodium metasilicate (SMS) is an alkaline antimicrobial compound approved by USDA for use in RTE meat and poultry products. Sodium metasilicate has been found to be effective against various foodborne pathogens, but the mechanism by which it inactivates foodborne pathogenic bacteria needs investigation.

Purpose: To determine the antimicrobial mechanism of action of sodium metasilicate for inactivation of *L. monocytogenes*.

Methods: The effect of SMS on the membrane integrity and viability of *L. monocytogenes* (Scott A) was determined by use of fluorescent propidium iodide (PI) and SYTO9 nucleic acid stains with subsequent flow cytometry. *Listeria monocytogenes* cells were treated with 2% SMS solution (w/v) and high pH (0.1 N NaOH) and stained with PI and SYTO9 and subjected to flow cytometry analysis to differentiate live and dead bacterial cells based on the membrane integrity. Transmission electron microscopy (TEM) was performed to observe the changes at cellular level following exposure of *L. monocytogenes* cell suspensions to 2% SMS and 0.1 N NaOH treatments.

Results: The disruption in membrane integrity was observed by uptake of PI by cells treated with SMS with subsequent flow cytometry. Ultrastructural changes from corresponding transmission electron micrographs further verified the disruption in the cytoplasmic membrane and changes in the morphology of the cells treated with SMS and high pH.

Significance: The results from flow cytometry analysis and transmission electron microscopy examination indicated that after SMS treatment, the membrane integrity of *L. monocytogenes* was compromised leading to leakage of intracellular contents and subsequent cell death.

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P2-102 Controlling *Aspergillus niger* on Strawberries by Recombinant Tobacco Osmotin for Extending Shelf-life

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Introduction: *Aspergillus niger* is one of the major causes of strawberry deterioration in the marketplace. Finding natural anti-fungal agents will help to extend the strawberry shelf life. Tobacco osmotin has been reported to have a wide range of anti-fungal activity.

Purpose: The purpose of this study was to control the growth of *Aspergillus niger* on strawberries by applying recombinant tobacco osmotin (rOSM) for extending the shelf life.

Methods: Recombinant tobacco osmotin was expressed in *Escherichia coli* and purified on cobalt-agarose affinity column. The inhibition on spore germination and mycelia growth of *Aspergillus niger* was tested. Strawberries were disinfected in 70% ethanol and rinsed in sterile water, and spot-inoculated with *Aspergillus niger* spores. Inoculated strawberries were dipped into rOSM solutions (350 to 1,400 µg/ml) for 1 min. After being air dried, strawberries were then stored at 4 and 25 °C for testing the anti-fungal activities. **Results** were compared using ANOVA.

Results: The inhibition of *Aspergillus niger* spore germination was 50% at 700 µg/ml concentration, and the mycelia growth was significantly inhibited. After 7 days stored at 25 °C, the reductions of strawberries infection in treated at 350, 700 and 1,400 µg/ml rOSM were 5, 10 and 20%, respectively compared to the control group (no rOSM treated). Strawberries in all treatments didn't show significant fungal growth at 4 °C after two weeks of storage.

Significance: This results showed promise for rOSM in controlling fungal growth. The rOSM has the potential to extend the shelf life of strawberries in the market.

P2-103 Citric Extracts Inhibit Motility, Biofilm Formation and Quorum Sensing in *Campylobacter jejuni*

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Developing Scientist Competitor

Introduction: The antimicrobial activity of citric fruits against *C. jejuni* has been demonstrated. Quorum Sensing (QS) a signaling system present in bacteria can influence the expression of a variety of virulence factors. Thus, interfering with QS represents an alternate mechanism for targeting virulence.

Purpose: This study was conducted to investigate the effect of citric extracts in the activity of AI-2 molecules that mediated QS in *Campylobacter jejuni* and their influence on motility and biofilm formation.

Methods: Cultures of *Campylobacter jejuni* were exposed to citric extracts (lemon peel, cider peel and orange peel) at different concentrations. For swarm motility test, sub-inhibitory concentrations of plant extracts were mixed with Muller Hinton with 0.3% agar. 5 µl of a *C. jejuni* culture were placed in the center of the plate and incubated for 48 h in microaerophilic conditions. The extent of swarming motility was determined by measuring the diameter of migration and compared with control. Biofilm formation in polyestirene microtiter plates with brucella broth was quantified after staining with safranin at O.D. 495 nm in a microplate reader. AI-2 activity was determined from supernatants of *C. jejuni* cultures in brucella broth; these were added into fresh culture (AB medium) of *V.harveyi* BB170. Culture supernatants of *V. harveyi* BB152 were used as positive control. The luminescence values were measured with a VictorX2 multilabel reader luminometer.

Results: Swarm motility was reduced 50% when treated with lemon peel or cider peel extract, and 35 to 40% for orange extract. Citric extracts reduced biofilm formation by 60 to 75% depending on extract concentration and /or strain tested. The AI-2 activity in supernatants treated with citric extracts decreased in most cases at levels of 90% Relative Light Units (RLU)

Significance: This study provides an insight of the effect of natural edible antimicrobials on QS and virulence factors of *Campylobacter jejuni*.

P2-104 Inhibition of Growth, Biofilm Formation and Swarming of *Salmonella* by Commercial Antimicrobial Citric Formulations

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Introduction: Clinical, outbreak-associated and retail product isolates of *Salmonella* show dense biofilm production, and swarming motility. Different natural antimicrobials based on citric extracts are commercially available, but little information is available on the effect on biofilm formation and swarming.

Purpose: Determine the efficacy of commercial natural antimicrobial formulations to control growth, biofilm production and swarming of *Salmonella*.

Methods: Different commercial formulations based on citric extracts were evaluated (Citric AB liquid, Citrik AB powder, Citrol K Ultra, Citrodex, Citrik Max and Citrik Agro). Minimal bactericidal concentrations (MBC) were evaluated against *S.* monophasic, *S.* Typhi, *S.* Typhimurium, *S.* Muenchen and *Salmonell*a spp. by microplate-dilution method. For swarm motility test, sub-inhibitory concentrations of formulations were mixed with LB with 0.5% agar. Five µl of bacteria cultures were placed in the center of the plate and incubated for 18 h. The extent of swarming motility was determined by measuring the diameter of migration and compared with control. Biofilm formation in polyestirene microtiter plates with TSB was quantified after staining with safranin at OD 492 nm in a microplate reader.

Results: MBC of formulations ranged from 81 to 922 mg/ml. Citrik AB was the most effective in inhibiting *Salmonella* growth (MBC 81-105 mg/ml). Citrodex showed the highest MBC (900 mg/ml). Most formulations inhibited biofilm formation at 75% of MBC, and a reduction was observed at lower concentrations. However, Citrik AB inhibited the biofilm formation even at 25% of the MBC, and also produced the higher swarming reduction (75%) when 75% of the MBC was added to treatments. The other compounds showed less swarming reduction.

Significance: These formulations could represent good alternatives to control the presence of *Salmonella* in circumstances were growth, biofilm formation and swarming are involved in survival or contamination.

P2-105 Survey of Antimicrobial Activity of Florida Honeys against *Staphylococcus aureus*

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Introduction: Honey has a history of being used as a topical antimicrobial agent. It has been shown that possible antimicrobial properties of honey are related to either hydrogen peroxide released from glucose upon action of glucose oxidase, an enzyme present in unprocessed

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honey, or non-peroxide-based activity, related to phytochemicals, such as methylglyoxal. New Zealand Manuka honey is widely known to possess antimicrobial activity, and is most effective against the pathogen *Staphylococcus aureus*.

Purpose: The purpose of the current study was to evaluate antimicrobial properties of 42 Florida honeys from different floral sources and geographical locations in their ability to inhibit growth of *Staphylococcus aureus* compared to commercially available Manuka honey and phenol standards.

Methods: The agar well diffusion method was used, and dilutions (50%) with deionized water (DI) or buffered peptone water (BPW), as well as treatment with catalase or autoclaving (to eliminate peroxide-based activity), were evaluated.

Results: Manuka honey antimicrobial activity was influenced by diluent, with inhibition zones of 8.7 ± 0.3 mm and 14.4 ± 0.7mm, for DI water (pH 4.18) and BPW (pH 5.82), respectively. Some Florida honeys (16/42) showed no inhibition, while 21/42 had activity equal to or superior compared to Manuka, with the rest showed less activity compared to Manuka control. Ten Florida honeys showed an average inhibition zone above 18 mm with standard deviations not exceeding ± 0.9 mm. Antimicrobial activity of Florida honeys was completely diminished after addition of catalase. Similarly, autoclaving reduced antimicrobial activity of all Florida honeys.

Significance: To summarize, we showed that significant fraction of Florida honeys possessed antistaphylococcal activity similar to or exceeding activity of commercially available Manuka honey, though the results of the catalase addition and autoclaving suggest the activity was possibly solely related to the presence of glucose oxidase.

P2-106 Influence of Extracts of Edible and Medicinal Plants on Membrane Damage and Expression of Enterotoxin of *Vibrio cholerae*

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Developing Scientist Competitor

Introduction: The search for natural antimicrobials to use in foods is encouraged by the high prevalence of foodborne diseases and the current popular preference of consuming all-natural foods. The mechanisms of action of most plant extracts with antimicrobial activity have been poorly studied.

Purpose: In this work, changes in membrane integrity, membrane potential, internal pH (pH_{in}), ATP synthesis and expression of the Cholera toxin gene, were determined in *V. cholerae* cells after exposure to extracts of edible and medicinal plants.

Methods: Preliminary antimicrobial properties of methanolic, ethanolic, and aqueous plant extracts were evaluated using the hole-plate diffusion technique. A micro-dilution method was used to determine the minimal bactericide concentrations (MBC) of extracts showing the higher antimicrobial activity. For the experiments, 10X MBC was used. Damage of *V. cholerae* membrane was evaluated using the LIVE/DEAD BacLight kit, cFDA-SE was used to measure internal pH, membrane potential was determined with $DiBAC_4(3)$ and ATP was measured using Enliten ATP detection kit. Expression of the Cholera toxin gene was determined by qRT-PCR.

Results: The methanolic extracts of basil, nopal cactus, sweet acacia, and white sagebrush, were the most active against *V. cholera*, with MBCs ranging from 0.5 to 3.0 mg/ml. Membrane integrity was affected by all selected extracts damaging 100% of the cells. The pHi showed a clear decrease from approximately pHi 7 to pHi 4. Membrane potential was affected; three plant extracts produced hiperpolarization and one extract depolarization. Cellular ATP production was reduced 55 to 89% depending on extract and strain tested. The expression of *V. cholerae* enterotoxin gene was not inhibited during the treatment. All extracts exerted profound physiological changes that lead to bacterial death.

Significance: These four plant extracts could be regarded as alternatives to control *V. cholerae* contamination in foods and in the treatment of diseases associated with this microorganism.

P2-107 Inhibitory Effect of Chitosan and Organic Acids on the Growth of *Listeria monocytogenes* in Ready-to-Eat Shrimp during Refrigerated Storage

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Introduction: The contamination of *Listeria monocytogenes* in ready-to-eat (RTE) shrimps has necessitated the application of effective post-lethality intervention strategies. Chitosan and organic acids are natural preservatives with proven antimicrobial efficacy; however, there is limited data on their combined use for control of *L. monocytogenes* in RTE shrimps.

Purpose: The study aimed to investigate the use of chitosan alone and combined with organic acids to reduce *L. monocytogenes* in RTE shrimps during refrigerated storage.

Methods: Cooked RTE shrimps inoculated with *L. monocytogenes* were dipped for 5 min in 0.5% and 1% (w/v) chitosan solutions and their combinations with 2% acetic acid (v/v), lactic acid (v/v), malic aicd (w/v) or citric acid (w/v), respectively. The treated samples were bagged and stored at 4 °C, and then bacterial counts and color and sensory properties were analyzed for a period of 20 days.

Results: The results indicated that 1% chitosan was more effective than 0.5% chitosan ($P < 0.05$) to inhibit bacterial growth, and their inhibitory effects were significantly enhanced ($P < 0.05$) when combined with 2% acetic acid, lactic acid or malic acid, but not citric acid ($P > 0.05$). The combination of 1% chitosan with 2% acetic acid was the most effective ($P < 0.05$) and caused 4.42 log CFU/g of bacterial reduction on 20 d when compared with controls, followed by the combined treatment of 1% chitosan with 2% malic acid or 2% lactic acid, respectively. The color and sensory characteristics of shrimps were not adversely affected by different treatment combinations ($P > 0.05$).

Significance: The study showed that the combined use of chitosan with organic acids could be a promising antimicrobial method to prevent the proliferation of *L. monocytogenes* in RTE shrimps.

P2-108 Evaluation of Sanitizers to Inactivate *Salmonella* on In-shell Pecans and Pecan Nutmeats

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Introduction: Although pecans have not been associated with outbreaks of foodborne illness, there have been USFDA recalls of pecans and a granola product containing pecans potentially contaminated with *Salmonella*. Little information on the effectiveness of sanitizers in killing *Salmonella* on in-shell nuts and nutmeats is available.

Purpose: The purpose of this study was to evaluate chlorinated water, organic acids (lactic, levulinic, peracetic) with and without sodium dodecyl sulfate (SDS), and water extracts of inedible pecan components for their efficacy in killing*Salmonella* on in-shell pecans and pecan nutmeats.

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Methods: In-shell pecans and nutmeats (USDA medium pieces) were dip inoculated in a five-serotype suspension of *Salmonella*, dried to ca. 3.7% moisture, and stored at 4 °C for 3 - 6 weeks. Samples were immersed in chlorinated water (200, 400, 1,000 µg/ml), lactic acid (0.5, 1, 2%) and levulinic acid (0.5, 1, 2%) with and without 0.05% SDS, a peracetic acid-based sanitizer (40 µg/ml), and water extracts of pecan hulls, shells, and pith at 21 °C for up to 20 min.

Results: Treatment of in-shell pecans with chlorine (400 µg/ml) for 20 min reduced *Salmonella* by 2.6 log CFU/g, whereas treatments with 2% lactic acid plus SDS and 2% levulinic acid plus SDS reduced the pathogen by 3.7 and 3.4 log CFU/g, respectively; treatment with the peracetic acid-based sanitizer resulted in a 2.4-log CFU/g reduction. Lactic and levulinic acids without SDS were less effective than acids with SDS. Reductions on nutmeat pieces were less than 1.1 log CFU/g, regardless of acid or acid/SDS concentration or treatment time. Compared to acid treatments, treatment with water extracts of inedible nut components was less effective in killing *Salmonella*.

Significance: None of the sanitizers evaluated was effective in killing high numbers of *Salmonella* (> 3.7 log CFU/g) on in-shell pecans and nutmeats. The importance of preventing contamination cannot be overemphasized.

P2-109 *Brucella* Identification and Speciation by Luminex Bead-based Suspension Array

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Introduction: Globally, unpasteurized milk and milk products are vehicles for the transmission of brucellosis, a prevalent zoonosis worldwide and foodborne illness in the United States. The intentional contamination of food with *Brucella*, a bio-threat agent, is also of serious concern. Although PCR assays do exist for the presence of *Brucella* spp., they are restricted by the resolution of band sizes on a gel or the number of fluorescent channels in a single real-time system. The Luminex bead-based suspension array is performed in a 96-well plate allowing for high throughput screening in minutes of up to 100 targets in one sample with easily discernible results.

Purpose: The purpose of this study was to develop a Luminex bead-based suspension array to identify *Brucella* at the genus level as well as *B. abortus*, *B. melitensis*, and *B. suis*.

Methods: A multiplex conventional PCR assay was designed to amplify *Brucella* DNA. The suspension array consists of uniquely colored fluorescent microspheres conjugated to specific probe DNA sequences for *Brucella* at both the genus and species level. Labeled PCR amplicons are coupled to the microspheres and analyzed by fluorescence readings through a flow cytometer (Bio-Plex 200 system). Signal-to-background ratios are calculated and samples were considered to be positive when signal to background ratio was greater than 6.0.

Results: The specificity of the assay was tested using at least 5 strains per target species. Signal-to-background ratios of 6 or greater (indicative of a positive sample) were achieved for all targets across all strains tested. Ratios ranged from 50 for most *B. abortus* strains tested to 26 for a genus-level target (16S FL). All probes showed high specificity, with no false positives (cross-reaction with non-target strains).

Significance: This high throughput assay provides a rapid (3-4 hours including PCR) and accurate sample screening tool for the detection of *Brucella* spp.

P2-110 Validation of the MWY Medium for Enumeration of *Legionella* in Water from Cooling Towers and Sanitary Hot Water

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Introduction: MWY (bioMerieux) is a selective agar for detection, enumeration and presumptive identification of *Legionella*. All species of *Legionella* grow producing grey-blue colonies.

Purpose: A study was conducted at the independent Expert Laboratory ISHA to validate this new method for detection and enumeration of *Legionella* in cooling towers and sanitary hot water, in comparison to the NF T90-431 and ISO 11731 reference methods and according to the NF Validation guidelines.

Methods: Water samples were analyzed following protocols of the references methods in which the MWY medium was compared to the GVPC medium. All presumptive-positive colonies were confirmed after streaking on BCYE (with and without L-cysteine) and then identified using the slidex *Legionella* kit.

Results: The MWY method showed satisfying relative linearity performances, with linear correlation coefficients superior to 0.95. Biases between both methods were characterized by low values. The intercepts close to 0 and the slopes close to 1 were validated for all the tested categories in the accuracy study. The selectivity and specificity of the MWY agar were shown to be good by testing 30 target and 30 non-target strains.

Significance: For all water evaluated, the MWY method demonstrated comparable results to the reference methods for enumeration of *Legionella*. Its higher selectivity reduces greatly the interfering bacterial growth and specially the fungal growth and facilitates the reading and the confirmation.

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P2-111 Polyphasic Approach for Quantitative Analysis of Obligately Heterofermentative *Lactobacillus* Species in Cheese

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Introduction: Obligately heterofermentative lactobacilli (OHL) present in cheese during ripening can influence the flavor and texture of the final product. In order to better evaluate, follow and control this population, there is a current need for easy-to-use tools.

Purpose: A culture-dependent quantitative method (ABEV) and a culture-independent real-time PCR (qPCR) assays was developed to follow OHL population dynamic from milk samples to the end of cheese ripening.

Methods: A selective and specific ABEV medium was developed for direct enumeration of total OHL from cheese, and a specific qPCR assays was set up to target *Lactobacillus fermentum* and *L. parabuchneri* individual species. These tools were applied for OHL quantification in manufactured Emmental and Tomme cheeses.

Results: The ABEV medium was well adapted for specific enumeration and isolation of OHL species present in milk-derived samples, even in the presence of background microbiota. qPCR assays showed 100% specificity and could accurately quantify the targeted species in various types of cheese. Culture-dependent and -independent techniques evaluated in manufactured cheese samples generated similar bacterial counts. The behavior of *L. fermentum* and *L. parabuchneri* was characterized during cheese processes. PCR-TTGE was also used to confirm the presence of inoculated species and to analyze the composition of naturally present species.

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Significance: Although the influence of OHL species during cheese ripening is recognized, information about their dynamic behaviour remains scarce due to the lack of simple analytical tools. This work illustrates how various culture-dependent and –independent methods can be implemented to study OHL, either as a population or as individual species. Both ABEV culture medium and qPCR tools present the advantage to be suitable for quantification of minor and dominant species and/or populations and are fully applicable to complex cheese ecosystems. A polyphasic approach combining these two quantitative methods with the more global PCR-TTGE technique showed their complementarity.

P2-112 An Improved Double Layer Plaque Assay for Male Specific Bacteriophage MS2

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Introduction: Plaque assay is an extremely important enumeration technique used in the study of bacteriophages. Traditional double layer plaque assay usually produces small and hazy plaques difficult to detect. A plaque assay with good visibility is critical to the accurate enumeration of bacteriophages.

Purpose: Develop an accurate enumeration technique for bacteriophage MS2.

Methods: An optimization of the double layer plaque assay for bacteriophage MS2 was developed. A double layer tryptone agar plate containing a thin layer (10 ml per 100 x 15 mm plate) of freshly prepared bottom agar (1%) with a thin layer (10 ml) of freshly prepared top agar (0.45%) was used for plaque enumeration. Both layers were supplemented with 0.1% glucose, 5 mM $CaCl_2$, 10 µg/ml thiamine, and the top layer also contains the 6-h culture of *E.coli*, the host of bacteriophage MS2. Thirty µl of bacteriophage MS2 stock was spread on top evenly and the plate was incubated at 37 °C for 12-15 h.

Results: The influences of bottom layer composition (w/, w/o supplements, 1%, 1.2% agar), bottom layer thickness (10, 20, 30 ml), top layer composition (w/, w/o supplements, 0.45%, 0.6% agar), top layer thickness (10 ml, 15 ml, 20 ml), position of bacteriophage (contained in top layer, spread on top), quantity of bacteriophage (30, 50, 100 µl) on the visibility of the plaques were investigated. Results indicated that adding supplements to both layers and decreasing top layer agar content extensively increase the plaque size. A combination of a thin bottom layer and a thin top layer produces the largest plaques. The plaque size is increased when the bacteriophage is spread on top instead of contained in the top layer.

Significance: The development of a novel double layer plaque assay ensures accurate detection and enumeration of bacteriophage MS2.

P2-113 Development of a Novel Polymerase Chain Reaction Electrospray Ionization Mass Spectrometer (PCR/ESI-MS) Assay for the Detection and Differentiation of Human Noroviruses

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Introduction: Norovirus is the leading cause of foodborne illness in the United States; however, current methods for differentiation of genotypes and strains are time-consuming and inconsistent. A novel method using PCR/ESI-MS may allow for detection and differentiation of noroviruses at the genotype and strain level within one working day. This method uses the exact weight of a PCR amplicon to determine its base composition and then compares this measurement against a reference database to identify the sample.

Purpose: The goal of this study was to develop a PCR/ESI-MS assay for the rapid detection and genetic differentiation of noroviruses.

Methods: To design primers for this assay, over 5,000 norovirus sequences were downloaded from GenBank. Sequences were aligned with BioEdit and primers were designed to amplify all the human noroviruses. Primers were analyzed*in silico* for parameters such as primer-dimer formation, % GC, and annealing temperature. Predicted base compositions related to each genotype were determined and used to select the final primer sets that provided maximal differentiation amongst the various genotypes.

Results: Following the initial design and *in silico* analysis of primers, a total of 8 primer pairs were selected for use in the PCR/ESI-MS assay that are arranged in a 96-well plate format, allowing for analysis of 12 samples per plate. These primer pairs amplify 50-150 bp fragments within the genes coding for the major capsid protein (VP1) and RNA-dependent RNA polymerase (RdRp). Analysis of the amplicons with ESI-MS is predicted to enable differentiation of 30 human norovirus genotypes, as well as strains within the GII.4 genotype.

Significance: The assay developed in this project will reduce the time and labor needed to detect and differentiate noroviruses and will enhance the ability of public health scientists to identify the source and the spread of norovirus illnesses related to food outbreak situations.

P2-114 Interpreting Marginally Positive RT-qPCR Results Derived from Naturally-contaminated Samples: What Does It all Mean?

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Introduction: Quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) have great potential to detect low levels of non-cultivable pathogens in food and environmental samples. Cloning and sequencing of amplicons is recommended for confirmation of presumptively positive results. Nonetheless, amplifications with cycle threshold (Ct) values above 35 are common, difficult to interpret, and virtually impossible to confirm by sequencing.

Purpose: To develop a DNA hybridization method to confirm presumptively positive RT-qPCR results obtained from environmental samples screened for evidence of human norovirus (HuNoV) contamination.

Methods: RT-PCR (traditional and quantitative) was applied to RNA extracted using a silica-based guanadinium isothionate method, followed by amplification using primers JJV2F and COG2R targeting the ORF1-ORF2 junction (region B) of the HuNoV genome. Probe Ring2P was used for qRT-PCR (45 cycles) and hybridization. After optimization of the hybridization, serial dilutions of stool samples positive for HuNoV were subjected to RT-qPCR and traditional RT-PCR followed by dot blot hybridization to compare assay detection limits. Both methods were applied to a subsample of 20 environmental samples collected from daycare facilities in the Carolinas previously tested for evidence of HuNoV contamination using RT-qPCR.

Results: As applied to serially diluted HuNoV-positive stool, RT-qPCR Ct values ranged from a low of 22 to a high of 38. Detection limit of the RT-qPCR method was identical to that of RT-PCR followed by hybridization. When both methods were applied to environmental samples all 10 samples previously screened negative for HuNoV by RT-qPCR were also negative by hybridization. However, 10 samples previously classified as presumptively positive for HuNoV based on Ct values ranging from 36-44 were in fact negative by hybridization.

Significance: The inability to confirm samples in the Ct range of 35-45 suggests cautious interpretation of RT-qPCR results and further highlights the need for confirmation. DNA hybridization is a viable method to confirm the status of such samples when sequencing is not possible.

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P2-115 Development of a New Strategy for Mapping Microbiomes of Food Manufacturing Facilities

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Introduction: Food manufacturing facilities are collections of microbial ecosystems with microbial members in harborage sites in equipment and the environment converging from diverse origins such as ingredients, air, dust, and employees. New culture-independent tools such as deep pyrosequencing provide a means to identify and quantify complex communities of microorganisms in a production setting, providing insight into the origins of these communities.

Purpose: One of the most significant issues in application of culture-independent PCR-based approaches is determining viability of taxa that are detected. In this study, a method was established and used to identify composition of viable microbes in communities from pre-kill portions of a thermal processing line.

Methods: Samples from ingredients, environmental swabs, and finished product were collected from a manufacturing facility. DNA was extracted directly from one portion (non-enriched) and another portion was inoculated into broth incubated at three different temperatures (enriched). DNA content from extracts of the "non-enriched" and "enriched" samples was measured by quantitative Real-Time PCR (qPCR) using the 16S ribosomal RNA gene (16S rDNA) as a target. Growth was measured independently by aerobic plate counts (APC) and by deep pyrosequencing of 16S rDNA amplicons from these same samples.

Results: At the optimal enrichment temperature of 35 °C, samples having enriched/unenriched 16S rDNA qPCR ratios >10 had viable cells as measured by APC while samples with ratios <10 had APCs ranging from undetectable to 100 CFU, establishing 100 CFU as a minimum viable cell threshold. As with APC, deep pyrosequencing of samples with enriched/unenriched qPCR ratios >10 were consistent with viable cells being present, showing large changes in the proportions of taxa detected after enrichment. Compositional analyses of sequences from sequential samples of a production line by linear discriminant analysis were consistent with viable organisms from ingredients and the environment converging to form communities on equipment prior to the thermal process step.

Significance: Optimization of this sample preparation and sample differentiation is an important first step in application of deep pyrosequencing to study the microbial ecology of food manufacturing facilities. We have used this method along with deep pyrosequencing to define composition of viable microbial communities in a manufacturing plant.

P2-116 Performances Assessment of a New Method According to the ISO 16140 Standard for the Next Day Detection of *Salmonella* in Foods, Feeds and Environmental Samples

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bioMerieux, Marcy L'Etoile, France

Introduction: The VIDAS® UP *Salmonella* (SPT) assay is a specific phage protein ligand assay performed in the automated VIDAS instrument associated with a 1-step enrichment procedure.

Purpose: A study was conducted by the independent Expert Laboratory Eurofins IPL Nord to validate this new method, as part of the NF Validation approval process.

Methods: Samples, were enriched for 18-24 hours at 41.5 ± 1 °C in Buffered Peptone Water with the addition of the *Salmonella* supplement within 0-45 minutes after stomaching. After incubation, samples were boiled for 5 ± 1 minutes before performing the assay. All presumptive-positive samples were further confirmed after streaking on a chromogenic agar plate. This new method was compared to the ISO 6579 reference method, according to the ISO 16140 standard.

Results: A comparative study was performed on 379 products distributed over the 6 categories meat, dairy, vegetable and seafood, miscellaneous products, feeds and environmental samples of which 26% were naturally contaminated. Similar results were obtained by both methods showing a sensitivity of 96.8% for the phage method and of 96.3% for the reference method.

The 50% detection limit was found to be between 0.3 and 1.3 CFU/25 g for the alternative method and 0.3 and 1.1 for the reference method. The collaborative study performed in 14 laboratories, showed a sensitivity of 88.4% and 92.1% for, respectively, the alternative method and the reference method. There was no statistical difference between the two methods using the Mc Nemar test at 5% level.

Significance: The SPT method was certified by AFNOR Certification to be equivalent to the reference ISO 6579 method for the detection of *Salmonella* in foods, feeds and environmental samples. The method is simple, fast and convenient and represents a very significant step forward in *Salmonella* testing, reducing the time for a result, as well as labour and media requirements

P2-117 Isolation and Detection of Pollen DNA in Honey

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Introduction: A recent decision from the European Court of Justice requires that honey containing pollen from GM plants be declared and receive authorization to be sold within the EU. As a consequence, in order to be imported into the EU, honey will need to be tested for the presence of genetic modifications in the pollen DNA. Honey provides challenges for DNA purification due to its high viscosity and sugar content and the varied, and generally low, amounts of pollen found in honey.

Purpose: The purpose of this study was to develop a method to isolate pollen DNA from honey and to determine the suitability of the DNA for use in a real-time PCR assay. Canola DNA primers and probes were used in a model real-time PCR detection system.

Methods: Samples of canola honey were dissolved in distilled water at a temperature of 30-40 °C. Pollen was centrifuged at 4000 x g for 10 minutes, resuspended in distilled water, aliquots pooled and recentrifuged for 4 minutes. The washed pollen was resuspended in QIAGEN Fast Lysis Buffer, and samples transferred to Lysis Tubes (Cat. No. 69534). Following overnight incubation, 200 µl of the sample in lysis buffer was used for DNA purification with the "Standard Protocol 200 mg" of the DNeasy *mericon*™ Food Kit (Catalog Number 69514) . A real-time PCR reaction containing the purified canola DNA and canola-specific primers was performed to determine the extraction efficiency of pollen DNA and its suitability for use in the *mericon* PCR assay portfolio.

Results: DNA purified from canola honey was shown to be detected in the real-time assay in a specific and efficient fashion by comparison to canola DNA standard curves using defined amounts of canola DNA. C_T of the standard curve dilutions were separated by 3 units over the range 25-34 C_T, and the canola DNA C_T was 29 + 0.7.

Significance: The DNA purification method presented can be used to reliably and specifically isolate DNA from honey for use in real-time PCR assays for detection of specific genes, such as those found in GM events, in a challenging food matrix.

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P2-118 Evaluation of Molecular Serotyping of *Salmonella* Using a New Multiplexing Technology

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Introduction: *Salmonella* infections are among the leading cause of enteric illness in the United States, with an estimated 1.4 million cases of salmonellosis occurring annually. Currently, there are over 2,400 recognized serovars of*Salmonella.* Rapid identification of *Salmonella* serotype would provide food producers valuable information that could be used to identify sources of *Salmonella* contamination.

Purpose: The purpose of this study was to evaluate the accuracy of a molecular serotyping method using known serovars of *Salmonella*.

Methods: DNA was extracted from 49 *Salmonella* isolates with known serotypes. Multiplex PCR was performed using Luminex O, H, and AT primer mixes according to the manufacturers instructions. The resulting PCR product was then hybridized with the microspheres specific to select gene targets and analyzed on the Luminex® 200™ instrument.

Results: Of the 49 isolates tested, the multiplexing *Salmonella* Serotyping Assay was expected to completely serotype 42 isolates and provide partial serotype information for the remaining 7. The multiplexing *Salmonella* Serotyping Assay correctly identified 42/49 isolates. Of the remaining 7 isolates, the assay narrowed the possible serotypes significantly. This would potentially reduce the time and expense associated with conventional agglutination serotyping to identify them.

Significance: These data suggest that molecular serotyping of *Salmonell*a using a new multiplexing technology can provide an accurate and rapid alternative to traditional agglutination serotyping.

P2-119 Rapid Extraction and Analysis of *Salmonella enterica* from Fish and Kitchen Surfaces

JESSICA CHAPMAN, Torrey Parrish, Megan Duggan
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Introduction: The demand for seafood is increasing globally, and currently in the United States, approximately 1/10 of imported seafood is rejected due to *Salmonella enterica* contamination. *Salmonella* is among the leading causes of foodborne illness caused by seafood. Currently slowed by plating or the combination of long extraction and analysis times, the need for rapid, on-site testing for this pathogen is essential to prevent the spread of disease.

Purpose: This test demonstrates successful *Salmonella* extraction from inoculated fish and kitchen surface swab using an Evogen ONE-UP extraction protocol and rapid LAMP amplification on the Optigene Genie II. The objective is to provide a combined rapid extraction and detection system.

Methods: Fresh fish samples were inoculated with 1ml *S. enterica* at 4,000 CFU/ml, or kitchen surfaces which had been in contact with the fresh fish were inoculated and dried for 30 minutes, then swabbed, and both swabs (in 1ml media) and fish samples (in 50ml media) were incubated at 37 °C for 24 hours. DNA from 200µl of each sample was extracted and purified by the Evogen ONE-UP extraction protocol, which included lysis with Evogen ONE, a bead-binding step, a wash step, and an elution step. Finally, eluted DNA was amplified via LAMP on an Optigene Genie II using primers targeting the *S. enterica* ttrRSBCA locus.

Results: All fish cultures showed amplification within 10 to 16 minutes on the Genie II, with the no template culture not showing any amplification. Similarly, fish surface swabs showed amplification within 10 to 13 minutes.

Significance: The combined system rapidly produced high quality DNA in that provided excellent amplification signals via LAMP. This combination of instruments demonstrates a simple and effective test method for detecting *Salmonella*that may easily be performed at any food testing or manufacturing facility.

P2-120 The Development and Validation of a Lateral Flow Device for the Rapid Detection of Total Milk Proteins in CIP and Environmental Samples

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Introduction: Allergy to bovine milk proteins represents one of the most common food-based allergies and cow's milk is one of the most common ingredients in food and nutritional supplements. Because of the widespread use of milk and milk proteins in food production, there is a need to screen for the presence of milk proteins in food handling areas and equipment**.** Reveal™3D Total Milk is a rapid lateral flow device which provides rapid and accurate information regarding presence of milk proteins.

Purpose: The purpose of this study was to develop and validate a new device for detection of milk proteins in clean-in-place (CIP) solutions and environmental samples and to determine the limit of detection of this assay.

Methods: A rapid lateral flow device for detection of total milk protein (including casein and whey) has been developed. This is a sandwich-based assay utilizing 1 antibody for capture and a 2nd antibody linked to a colored particle for detection. An extraction process has been developed to allow for detection of milk protein in various sample types including CIP and environmental samples.

Results: The device is accurate, rapid and simple and does not require special tools or extensive training and yields results in less than ten minutes from sample collection. The lower limit of detection is 5 ppm in CIP solutions and 20 µg/100 cm^2 for total milk proteins. This lateral flow device has a third line of detection, which is included to signal the presence of a grossly contaminated solution or surface and eliminate potential for false negative results in these samples.

Significance: This rapid lateral flow device provides rapid and accurate indication of the presence of milk proteins in CIP and environmental samples while providing confidence that highly contaminated samples are detected and not read as a false negative.

P2-121 Validation of a *Campylobacter* Real-Time PCR Assay for the Detection of *Campylobacter* in Chicken Carcass Rinses, Turkey Carcass Sponges and Raw Ground Chicken after a 24 h Enrichment

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Introduction: *Campylobacter* has emerged as the most frequent cause of gastroenteritis in humans. *C. jejuni*, *C. coli*, and *C. lari* are the species most commonly identified as causing infection. The majority of *Campylobacter* infections are acquired through the consumption of contaminated water, raw and inadequately pasteurized milk, and undercooked meats, particularly poultry. Surveillance through FoodNet indicates that over 2.4 million people are infected by *Campylobacter* each year.

Purpose: iQ-Check *Campylobacter* is a real-time PCR assay for detection of *C. jejuni*, *C. coli*, and *C. lari* in chicken carcass rinses, turkey carcass sponges and raw ground chicken. The data presented is the AOAC Performance Tested Method validation study for this assay.

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Methods: Inclusivity and exclusivity tests were performed to determine sensitivity and specificity of the assay. The proposed method was compared to the USDA MLG reference method for the analysis of chicken carcass rinses and turkey sponges and to the ISO reference method for the analysis of raw ground chicken. Chicken rinses and turkey sponges were naturally contaminated and two lots of each matrix were tested. Raw ground chicken was inoculated at two levels: a low level at 0.619 CFU/25g and a high level at 6.19 CFU/25g.

Results: Inclusivity and exclusivity testing yielded 100% accuracy. When food matrices were tested, all samples that were detected by the new method were subsequently confirmed by reference method protocol. There was no significant difference in the number of positive samples detected by the proposed *Campylobacter* kit in comparison to the USDA or ISO method for all matrices tested.

Significance: The new *Campylobacter* kit can be used after a single 24-hr primary enrichment. The culture method requires a 48-hr enrichment followed by another 48 hrs on a selective agar plate. The iQ-Check kit uses primers and specific DNA hybridization probes targeting a specific sequence on the *Campylobacter* genome. Detecting target DNA is possible even if the cells are stressed or in lower numbers. This increased sensitivity and time saving provide users with an alternative to the reference method for detection of *Campylobacter* in food samples to meet current regulatory demands.

P2-122 Alternative Quick Method for the Microbial Evaluation of UHT Soy Milk and Low pH Soy Milk Products

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Introduction: Microbiological evaluation of UHT aseptic products is aimed to verify commercial sterility after incubation of a statistically suitable number of samples. Rapid technologies, such as Bioluminescence have focused on detecting any microbial contamination in shorter times, allowing a faster release of the products and financial improvements to the Business.

Purpose: The objective of this study was to evaluate a new application of 3M™ Microbial Luminescence System (MLSII) with soy-based UHT products, including those with neutral (7.0-7.2) and low (3.8-4.0) pH.

Methods: 480 samples of soy-based products, including neutral and acidic ones, were artificially contaminated with very low levels of microorganisms (< 1 CFU/ml). 4 microorganism groups were used – *Enterobacteriaceae* (*E.coli*), *Bacillus*(*B.coagulans, B.stearothermophyllus, B.subtilis), Lactobacillus* (*L.plantarum, L.casei)* and Yeasts (*Picchia anómala, Saccharomyces cerevisiae*). Contaminated samples were incubated 48 and 72 hours at 32-35 °C and analyzed in duplicate by standard plate count method and the new method to detect microbiological growth. Six hundred samples from both product categories were tested to establish background levels of product ATP.

Results: Results show that reagents used for bioluminescence detection were capable of reducing the product ATP of samples to average levels of 10-20 RLU. Bacteria on artificially-contaminated neutral samples were detected after 48 hours of incubation and for acid samples the detection was at 72 hours for *E.coli.* However, acid samples contaminated with *Bacillus* didn't develop any growth when incubated up to 10 days. Samples contaminated with yeasts were detected after 72 hours for both types of products. All results obtained with the new method corresponded with positive samples in standard plate count method.

Significance: The new method is a reliable alternative for detecting microbiological contamination in aseptic non-dairy products. Bioluminescence system allows quicker and reliable results compared to traditional microbiological methods and pH testing as some contaminated samples didn't present any significant pH variation.

P2-123 Evaluation of a New Molecular Testing Method for Food Pathogens

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Introduction: Food Safety as an objective of the food industry ensures that manufactured products would not represent any health risk to consumers. Detection and identification of pathogenic microorganisms by traditional microbiology is time consuming, therefore is critical to adopt new and reliable technologies that can reduce time to results and increase sample analysis thus allowing for faster corrective actions and to generate food safety indicators to further help food manufacturers measure productive processes reliability.

Purpose: This study was conducted to evaluate the performance of the new 3M™ Molecular Detection System for implementation in routine testing of ready-to-eat foods. The novel method combines isothermal DNA amplification and bioluminescence detection as an alternative detection method for pathogens.

Methods: A total of 78 ready to eat food samples were artificially inoculated with strains of *S. enteritidis*, *L. monocytogenes* or *E.coli* O157 and 32 non contaminated samples were used as negative controls. All samples were enriched and incubated as follows: 18-24hours at 37°C for *Salmonella spp*, 40-48hours at 37°C for *Listeria spp* and 18-24 hours at 41.5°C for *E. coli* O157. All samples including negative controls were analyzed using the new detection system. Negative controls were confirmed by traditional methodology.

Results: All contaminated samples were detected by the new detection system as positive in less time than traditional methods. A matrix control reagent was used to verify inhibition of the amplification resulting in valid results. Non contaminated samples were detected as negative with the system and the absence of the pathogen was confirmed by traditional microbiology.

Significance: The new detection system was not only a suitable but also practical and sensitive method to detect the absence and/or presence of pathogens in a variety of RTE products when compared to traditional methods in a much shorter time.

P2-124 Validation of a New Molecular Detection System for the Detection of *Listeria* in Meat, Seafood, Dairy and Retail Environments

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Introduction: There is a continued need to develop improved rapid methods for detection of foodborne pathogens in the food processing environment, raw materials, and finished products. The 3M™ Molecular Detection System utilizes isothermal DNA amplification and bioluminescence to detect targeted pathogens, after an enrichment step.

Purpose: The project aim was to evaluate the new molecular detection assay for *Listeria* and to compare turnaround time and sensitivity to FDA-BAM standard methods, using environmental samples obtained from retail delis and meat, seafood, and dairy processing plants.

Methods: Environmental samples were collected using sponge-sticks with D/E Neutralizing Broth and tested for *Listeria* with the respective FDA-BAM standard methods and the 3M molecular detection system after 22 and 48 h enrichment in new modified *Listeria* Recovery Broth. Isolates from positive samples were confirmed by PCR; sequencing of the partial *sigB* gene was performed to characterize *Listeria*.

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Results: Among the 341 samples tested for *Listeria,* 72 were positive by the new molecular detection system and FDA-BAM; 262 were negative by both methods; 2 were positive by the molecular detection system and negative by FDA-BAM (enrichments were retested using the molecular detection system and plated to LMPM and MOX for confirmation); and one sample was negative by the molecular detection system and positive by FDA-BAM. There were no significant differences between *Listeria*-positive samples detected by the molecular detection system and FDA-BAM standard methods ($P = 0.945$).

Significance: Overall, the 3M Molecular Detection System shows promise for application for rapid testing of environmental samples.

P2-125 Rapid Detection of Botulinum Neurotoxin Activity in Food Matrices

WARD TUCKER
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Introduction: The mouse bioassay is the standard for botulinum neurotoxin (BoNT) detection in food samples in both government and industrial laboratories. While sensitive, the bioassay is low throughput, inaccurate, time consuming, and ethically challenging. Modern, biochemical methods are needed to replace animal-based methods for food challenge studies and food-related diagnostics. The BoTest™ Matrix Assays were developed by the detection botulinum of BoNT activity in complex matrices, offering sensitive, high throughput alternatives to current mouse bioassay methods.

Purpose: The assays were validated for the detection of BoNT in a range of food products including liquid and solid materials. These studies were used to establish the sensitivity of the assays in various matrices and to determine inter- and intra-assay variability.

Methods: BoNT/A, B, and F were spiked directly into food samples. Samples were then processed and subjected to the serotype-specific BoTest™ Matrix Assay. BoNT activity was quantified and results analyzed by BoNT serotype and food product.

Results: Limits of detection in 200 µL samples containing BoNT below 100 fM are possible with most tested matrices and below 10 pM for all sample types depending on the BoNT serotype and food product. Statistical analysis demonstrates intra- and inter-assay coefficients of variance below 10%. The assays were time-tunable and total assay times of less than 4 hours are possible depending on the desired sensitivity.

Significance: The BoTest™ Matrix Assays are rapid, robust, and high throughput alternatives to animal-based assays for BoNT potency testing and food challenge studies.

P2-126 Molecular Detection of *Escherichia coli* and *Salmonella* spp. in Contaminated Ground Meat with Immuno-magnetic Beads and Loopamp Kit

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Introduction: In the United States, sources of contaminated food is a major problem in affecting human health. The major contaminating organisms are *E. coli*-O157 and *Salmonella* spp., which are known to cause severe health complications like nausea, vomiting, stomach cramps and bloody diarrhea. A major requirement and challenge for the food industry is the rapid and accurate detection and identification of contaminating bacteria in food.

Purpose: Conventional "standard" methods are usually time consuming, require a minimum of 48 hours for detection and pose a threat for food industry in releasing products into the market. There is a great need for rapid food testing methods in the market that can efficiently detect contaminating bacteria. This modified method makes use of immuno-magnetic capture of contaminating bacteria in food and high sensitive detection using the LoopAmp kit for *E. coli*-O157 and*Salmonella* spp.

Methods: Ground turkey and ground beef from local stores were inoculated with concentrations 10^1, 10^2 and 10^3 CFU/ml of *E. coli*-O157, *Salmonella* spp., or both. Samples were collected at 2 hrs, 4 hrs and 6 hrs after incubation in mTSB media at 42 °C. The samples are concentrated using immuno-magnetic beads, extracted and amplified with LoopAmp kit. Our current 6 hrs enrichment protocol with LoopAmp kit is currently in AOAC review and is considered as standard in this study.

Results: The LoopAmp results were comparable to USDA and FDA standard methods and shorten the "time-to-result" in detection. In ground meat samples, we detected *E. coli*-O157and *Salmonella* spp., to as low as 10^1 CFU/ml with 6 hrs enrichment samples. Using improved method with magnetic beads, we can detect as low as 10^1 CFU/ml in 4 hrs and 10^3 CFU/ml in 2 hrs enrichment samples.

Significance: The use of magnetic beads in food samples has shown to produce sensitive and comparable results as with 6 hrs enrichment samples. Unlike most methods that require 8 hrs - 16 hrs enrichment time, this method can be used with a minimum 4 hrs enrichment time. This study has a great potential in rapid food testing and provides highly sensitive and comparable results with less "time-to-result."

P2-127 Performance Assessment of a Real-Time PCR Method According to the ISO 16140 Standard for *Listeria monocytogenes* Detection in Food and Environmental Samples

Justine Baguet, Muriel Bernard, Cecile Bernez, Claudie Le Doeuff, Sarah Peron, Maryse Rannou, DANIELE SOHIER
ADRIA, Quimper, France

Introduction: The MicroSeq® *L. monocytogenes* detection kit is a new detection method based on real-time PCR principle. The users have the flexibility to choose between the manual Rapid Spin protocol and the automated MagMax protocol.

Purpose: An independent study was conducted to validate this new method in comparison to the ISO 11290-1 reference method, as part of the NF Validation approval process and according to the ISO 16140 standard. The Rapid Spin and the MagMax protocols were both tested.

Methods: The Rapid Spin protocol includes a single enrichment step in Half-Fraser broth, while the MagMax protocol requires an additional sub-culture in Fraser 1. After DNA extraction, the MicroSeq® *L. monocytogenes* Real Time PCR is run with the 7500 Fast Automate.

Results: 407 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the real-time PCR method and the ISO 11290-1 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the real-time PCR method vary from 0.3 et 1.6 CFU/25 g, those of the ISO standard vary from 0.2 to 1.1 CFU/25 g. The selectivity and specificity of the alternative method was assessed by testing 50 target strains and 30 non-target strains. The alternative method was also evaluated in a ring trial involving 17 laboratories. The results of the calculated accuracy, accordance, concordance, and odds ratio clearly show that the real-time PCR method precision is equivalent to the ISO 11290-1 standard one.

Significance: The real-time PCR method is a reliable alternative method for *L. monocytogenes* detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

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P2-128 Development of a Rapid Method for the Detection of *Salmonella* in Environmental Samples of the Primary Production Stage (PPS)

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bioMerieux, Marcy L'Etoile, France

Introduction: The development of a rapid method for *Salmonella* detection in PPS samples (e.g., pig and poultry feces) is extremely challenging because of their complex and diverse nature, high levels of background flora and the presence of inhibitors.

Purpose: The objective of this work was to define and to evaluate an enrichment protocol suitable for PPS samples allowing the next day detection of *Salmonella* with the assay.

Methods: Faecal samples from pigs and poultry were enriched for 16 to 24 h at 41.5 °C in buffered peptone water supplemented with different selective agents, tested at different concentrations, prior to performing the assay. Transfer to a selective broth, *Salmonella* Xpress2 broth (SX2) was also studied with an incubation period between 6 and 24 hours at 41.5 °C. Positive results were confirmed by streaking the broths onto selective agar. The ISO 6579/A1 method was used as the reference method.

Results: Among all the supplements tested, the proprietary *Salmonella* supplement provided the best recovery of *Salmonella* but at double the concentration used for analysis of food samples. A transfer into the selective SX2 broth was also necessary to avoid false-negative results. A transfer of 1 ml into 10 ml of SX2 broth gave better results than 0.1 ml or even 0.5 ml, and incubation for 6 hours at 41.5 °C was comparable to 24 hours. Of the 154 products tested, the new method provided 81 positive results compared to 69 for the reference method. Overgrowth of plates by competing flora was an issue and the SALSA plate gave the best recovery. In some cases, a second transfer in SX2 broth before streaking, was necessary for confirmation.

Significance: The new enrichment protocol for the *Salmonella* assay provided better results than the ISO 6579/A1 reference method for the detection of *Salmonella* in environmental samples of the PPS, allowing release of negative products in less than 26 hours.

P2-129 Thermal Inactivation of Shiga Toxin-producing O157:H7 (ECOH) and Non-O157:H7 Shiga Toxin-producing (STEC) *Escherichia coli* Cells in Wafers of Ground Beef

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Introduction: ECOH and six other highly pathogenic serotypes of STEC are adulterants in ground/non-intact raw beef. However, it is not known if validated thermal interventions for ECOH in raw beef would also be effective for inactivating STEC.

Purpose: Quantify the effect of heat on the fate of ECOH and STEC in a model beef system.

Methods: For each of two trials, freshly-ground beef of higher (70:30 = percent lean:fat) or lower (93:7 = percent lean:fat) fat content was inoculated separately with a single strain of *E. coli* serotypes O111:H-, O45:H2, O103:H2, O121:H19, O145:NM, O26:H11, and O157:H7 to ca. 7.0 log CFU/g. Next, 3-g samples were transferred into filter bags. With the aid of a stainless-steel roller, the meat was flattened (ca. 1.0 mm) within the bags and then vacuum-sealed. For each target temperature and time point, three bags were submerged in a thermostatically-controlled water bath and heated to an internal temperature of 54.4 °C for up to 90 min, or 60 °C for up to 4 min, or 65.6 °C for up to 0.5 min. Note, the safe cooking guidance for raw beef products generally is above 62.8 °C.

Results: D-values decreased as the heating temperature increased. In lower fat wafers, D-values ranged from 13.7 to 23.8 min, 0.86 to 1.33 min, and 0.19 to 0.22 min at 54.4 °C, 60 °C, and 65.6 °C, respectively. Heating higher fat wafers to 54.4 °C, 60 °C, or 65.6 °C generated D-values of 20.4 to 32.7 min, 0.83 to 1.22 min, and 0.18 to 0.34 min, respectively. In general, with the exception of wafers heated to 54.4 °C, no effect of fat level on lethality of STEC and ECOH and no appreciable differences in thermal inactivation among STEC serotypes and between STEC and ECOH were observed.

Significance: These data established that cooking times/temperatures effective for inactivating *ECOH* in ground beef are equally effective against the additional *STEC* strains investigated.

P2-130 Terahertz Time-domain Spectroscopic Analysis of Zearalenone and Its Derivatives

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Introduction: The terahertz (THz) frequency region is located in the electromagnetic spectrum between microwaves and the infrared, and corresponds to photon energies between 1 and 100 meV. Owing to the highly collective character of low frequency vibrational modes, it is shown that most of chemical materials can be distinguished by monitoring their THz spectral fingerprints. However, feasibility studies for application in the food sector are very limited.

Purpose: The aim of this study is to provide data of the spectral characteristics in the THz region of zearalenone (ZEN), a potent oestrogenic mycotoxin, and its derivatives. The potential application of THz time-domain spectroscopy was further investigated by evaluating the detection feasibility.

Methods: The absorption spectra and refractive indices of ZEN and its five derivatives (zearalanone, alpha-zearalenol, alpha-zearalanol, beta-zearalenol and beta-zearalanol) were measured in the range from 0.1 to 2.5 THz using THz time-domain spectroscopy. Computational chemistry using the density functional method was used to study structure and internal rotations in ZEN.

Results: The spectrum of ZEN in the solid phase displayed several absorption peaks over frequency range from 0.1 to 2.5 THz, with significant peaks observed at 1.6, 2.2 and 2.8 THz. Among five derivatives, zearalanone and beta-zearalanol showed specific fingerprints in the frequency range between 0.1 and 2 THz. Theoretical simulation of ZEN shows that the distinct features of the spectrum originated from low-frequency vibrational modes caused by intra-molecular collective motion. The specific absorption peaks of ZEN, zearalanone and beta-zearalanol were still identifiable when mixed with wheat flour, and showed strong intensity in proportion to the concentration (0.1 ~ 10%).

Significance: These results provide the experimental proof-of-concept that THz time-domain spectroscopy could be suitable for screening applications of ZEN and its derivatives in food.

P2-131 Performance Assessment of a New Method for *Cronobacter* spp. Detection

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Introduction: The RAPID'Sakazakii method is a new *Cronobacter* spp detection method based on a chromogenic principle.

Purpose: An independent study was conducted to validate this new method in comparison to the ISO/TS 22964 reference method, as part of the NF Validation approval process and according to the ISO 16140 standard.

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Methods: The protocol includes an overnight enrichment in BPW. The characteristic colonies are confirmed by running oxidase tests and biochemical galleries. Flexibility is offered by storing the enrichment broth at 5 ± 3 °C for 3 days, before streaking onto the selective agar.

Results: Seventy-seven infant formula samples were analyzed in the relative accuracy, sensitivity and specificity study, which concludes to equivalent performances between the new method and the ISO/TS 22964 methods. The relative detection limits of the new method and the ISO/TS 22964 methods are clearly similar and vary from 0.8 et 1.2 CFU/25 g. The selectivity and specificity of the alternative method was assessed by testing 52 target strains and 31 non-target strains.

Significance: The new method is a reliable alternative method for *Cronobacter* spp detection in infant formula, and offers important economic savings by reducing time to result and handling time.

P2-132 Performance Assessment of New Real-Time PCR *Listeria* spp. Detection Kit According to the ISO 16140 Standard for *Listeria* spp. Detection in Food and Environmental Samples

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ADRIA, Quimper, France

Introduction: The MicroSeq® *Listeria* spp detection kit is a new detection method based on real time PCR principle. The users have the flexibility to choose between the manual Rapid Spin protocol and the automated MagMax protocol.

Purpose: An independent study was conducted, to validate this new method in comparison to the ISO 11290-1 reference method, as part of the NF Validation approval process and according to the ISO 16140 standard. The rapid and automated protocols were both tested.

Methods: The rapid protocol includes a single enrichment step in Half-Fraser broth, while the automated protocol requires an additional sub-culture in Fraser 1. After DNA extraction, the new real time PCR is run with the 7500 Fast Automate.

Results: 398 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity. The results demonstrate equivalent performances between the new real-tme PCR and the ISO 11290-1 methods. The relative detection limits of the new method vary from 0.3 to 1.6 CFU/25 g, those of the ISO standard vary from 0.2 to 1.2 CFU/25 g. The selectivity and specificity of the alternative method was assessed by testing 55 target strains and 32 non-target strains. The alternative method was also evaluated in a ring trial involving 17 laboratories. The results of the calculated accuracy, accordance, concordance, and odds ratio clearly show that the alternative precision is equivalent to the ISO 11290-1 standard one.

Significance: The proposed method is a reliable alternative method for *Listeria* spp detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

P2-133 Verification of Foodproof EHEC Screening by Real-Time PCR

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Introduction: Foodborne illness caused by Enterohaemorrhagic *Escherichia coli* (EHEC) claimed at least 50 lives during one of the largest outbreaks in 2011. EHEC have been encountered in leafy vegetables, sprouted seeds, raw milk and cheeses, as well as fresh, minced, and mixed meat preparations. Most infections have been with *E. coli* O157. However, there are various other strains of non-O157 *E. coli* capable of causing sickness and death that should be examined in food. Screening for pathogenic *E. coli* has been recommended using the virulence factors *stx*1, *stx*2, and the *eae* gene. Serotype *E. coli* O104:H4, the cause of this severe outbreak in 2011, could have been detected if such screening had been in place.

Purpose: Used during the outbreak of 2011 by German government laboratories, BIOTECON Diagnostics developed the foodproof EHEC Screening Kit to determine whether EHEC are present in a food sample in less than 24 hours.

Methods: This real-time PCR method is performed by separately detecting Shiga toxins *stx*1 and *stx*2 as well as the intimin *eae* gene in one simultaneous assay.

Results: Unlike the current ISO method, all known variations of the pathogenicity genes *stx*1, *stx*2, and *eae* can be detected with the foodproof EHEC Screening Kit, including *stx*2f. Following one enrichment, each kit achieves a sensitivity of detecting one cell in a food sample. Similar to other foodproof real-time PCR detection kits, BIOTECON Diagnostics has included additional controls to prevent false-positives and false-negatives. These kits are compatible with nearly all open real-time PCR instruments.

Significance: The foodproof EHEC Screening Kit can be used in conjunction with the foodproof *E. coli* O157 Detection Kit as well as the foodproof *E. coli* O104 Detection Kit to specifically identify these serotypes.

P2-134 Development and Testing of a Rapid Protocol for Environmental Swabs Using an Oxygen-Depletion Technology

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Introduction: It is common practice for the equipment and surfaces used in food processing and preparation to be tested for sterility by swabbing. Usually, the recovered microbial load from the swab is submitted for aerobic plate count (APC) testing using either the BAM or ISO methods. The plating methods and subsequent counting activities are manual and prone to error.

Purpose: A new technology has been introduced that can reduce the testing and enumeration time for even low bacterial loads on swabs, to less than 12 hours. A protocol using commercially available swab products has been developed that uses this oxygen depletion technology to reduce preparation time and also testing time while reducing errors.

Methods: In a three-phase program, the protocol was developed to determine the usefulness and typical processing time for swab samples. In Phase One the amount of microbial recovery into the sensing medium was estimated. In Phase Two, sterility validation tests were run in a typical food testing laboratory. Finally, a field trial was conducted in collaboration with a food processor.

Results: Estimated bacterial recovery from the swabs are presented. An instrument calibration curve is determined and statistical results from the lab and field trials are tabulated. 1) Bacterial recovery from selected swabs is confirmed to exceed 80%; 2) Correlation to the reference method in a controlled test is confirmed to be greater than 0.92; 3) Correlation to the reference method in a field study is confirmed to be greater than 0.92; and 4) Time to result is confirmed to be reduced by 75% versus the reference method.

Significance: This new method reduces the waiting time for swab results while also reducing the amount of lab preparation and time spent determining results, giving a food processor more control and improving food safety.

Poster

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P2-135 *Withdrawn*

P2-136 Development and Evaluation of a Real-Time PCR Assay for *Salmonella* Detection

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Introduction: *Salmonella* is found in many food and environmental sources, and it can cause serious illness. In the presence of competing flora, culture isolation may require lengthy procedures with skilled interpretation. Rapid, non-culture methods, such as those based on PCR, can provide for fast and accurate detection. The incorporation of real-time PCR detection into the current commercial BAX® System assay shortens the overall time to result with no loss in performance.

Purpose: The purpose of this study was to characterize the performance of the newly developed real-time PCR assay for genus *Salmonella* detection. Inclusivity and exclusivity of the new assay were compared to the commercial *Salmonella*assay, and sensitivity was determined in the presence of a variety of food types.

Methods: Studies evaluating the sensitivity of the new real-time assay were conducted using titrations of target cells inoculated into negative food enrichments. Cell lysates were also used to conduct inclusivity and exclusivity studies for the new real-time assay.

Results: The sensitivity of the assay was determined to be $1X10^4$ cfu/ml for each of the six *Salmonella enterica* subgenera as well as the species *Salmonella bongori*. The assay was 100% inclusive for all 409 *Salmonella* strains tested and 100% exclusive for all 44 non-*Salmonella* bacteria tested.

Significance: The new real-time PCR assay for *Salmonella* provides reduced processing time (65 minutes) with no change in sensitivity, inclusivity or exclusivity performance. This improved productivity will allow food manufacturers to make earlier, confident decisions regarding *Salmonella* presence in their products.

P2-137 Effect of Habituation on Plastic or Metal Surfaces in the Presence of Various Food Residues on Survival and Growth of *Salmonella*

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Developing Scientist Competitor

Introduction: Exposure of cells to various stresses during passage from foods to sponges and then to food containers (i.e., detergents, starvation on container surface, acids in food residues) may likely affect the growth dynamics of pathogens and, hence impact the risk of foodborne diseases.

Purpose: To evaluate the effect of the material of food containers in combination with the type of the food residues on; (i) the recovery of *Salmonella* sp. from surfaces and (ii) the growth rate and the lag time of *Salmonella* detached from surfaces and subsequently exposed to mild pH and NaCl conditions.

Methods: A 5-strain *Salmonella* composite was inoculated (6 log CFU/ml) in 5 food homogenates (FH; 1:10 with water); ground beef (GB), lettuce (L), mayonnaise (M), GB+L, GB+L+M, with or without 1% of a commercial detergent, while MRD served as control. Following incubation at 25 °C for 24h, plastic or metallic coupons (n=6) were spot-inoculated and stored under the same conditions. Cells were detached from each surface with a cotton swab. Growth kinetic parameters were determined in TSB with pH 5.5 and 2% NaCl in a Bioscreen C, by correlating serial dilutions with time to detection (TTD).

Results: *Salmonella* populations reached 7.9-8.5 log CFU/ml during storage in all FH with or without detergent, except for M where the low pH (3.93-4.08) caused slight reductions (0.3-1.7 log CFU/ml). The presence of the detergent resulted in lower recovery (0.3-2.5 log CFU/g) of the pathogen, regardless of the FH type or the material of the coupon. The growth rate of *Salmonella* (0.04 – 0.08 h^{-1}) was not significantly ($P > 0.05$) affected by the stresses encountered during storage on the coupons. In contrast, the lag time decreased ($P < 0.05$) by 2.7 hours and increased ($P < 0.05$) by 5.2 hours, following inoculation in V and M+V without detergent and remaining on metallic surface, respectively.

Significance: Results may assist to identify sources of contamination where risk is higher, and contribute with data on bacterial transfer for QMRA.

P2-138 Heat Tolerance of Shiga-toxigenic *Escherichia coli* (STEC) in Laboratory Media

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Introduction: Because of the USDA's new action classifying non-O157 STECs as adulterants in ground beef, it is imperative that the efficacy of cooking temperatures be determined on STEC O groups. It cannot be assumed the cooking protocols will effectively reduce non-O157 STECs if heat tolerance among strains exists.

Purpose: The objective of this research is to determine the efficacy of heat treatments on *Escherichia coli* O157:H7, O145, O45, O26, O111, O103, and O121 in Tryptic Soy Broth.

Methods: Tryptic Soy Broth inoculated with individual strains of STEC (target 108 – 109 cfu/mL) were subjected to three separate heat treatments mimicking cooking temperatures for beef (60, 65.5, and 71 °C). Effectiveness of heat treatment was measured in 30 s increments for several minutes. Samples were plated onto Tryptic Soy Agar and incubated at 37°C for 24 hours. The reduction in log cfu/mL was measured over time.

Results: The O145 and O45 serogroups were more heat stable while the O26 and O111 serogroups appear heat labile. At 60°C, O157:H7 was reduced by 6.5 log cycles. However, O145 and O45 were reduced by 2.2 and 1.4 log cycles. The O45 serogroup had a decreased reduction compared to O157:H7 at all temperatures, while O145 had decreased reductions at 60 and 71°C. At 65.5°C, O157:H7 was reduced 6.07 log cycles. All serogroups were reduced comparably with the exception of O111 which was reduced 10.9 log cycles. For the 71°C temperature, O157:H7 was reduced 6.41 log cycles. The O26 serotype did not tolerate heat treatment and experienced a 10.37 log cycle reduction.

Significance: Consumers follow the USDA recommended minimum internal cooking temperatures in order to effectively reduce E. coli O157:H7 and this research illustrates that some O groups survive temperatures that typically eliminate O157 in a broth model. Further research is needed to determine if the same reduction in pathogen load is observed when the bacteria are added to beef.

Poster

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P2-139 Prevalence and Characterization of *Salmonella* Serovars in Retail Ground Pork and Beef

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Developing Scientist Competitor

Introduction: *Salmonella* is a leading cause of foodborne disease worldwide. Outbreaks are frequently linked to the consumption of animal origin foods.

Purpose: To determine and characterize current *Salmonella* serovars present in ground meat and pork.

Methods: A total of 480 samples were collected (231 pork and 249 beef) from March 2009 to March 2010 from grocery stores located in the metropolitan area of Washington DC. Confirmed *Salmonella* isolates were characterized using serotyping, antimicrobial susceptibility testing and pulse field gel electrophoresis (PFGE).

Results: *Salmonella* was isolated from 3.6% (9) and 5.6% (13) of ground beef and ground pork samples, respectively. While the majority of strains were serotyped as *Salmonella* Typhimurium (10/29), eight other serotypes were also identified: *S.* Derbi, *S.* Infantis, *S.* Anatum, *S.* Dublin, *S.* Thompson, *S.* Johannesburg, *S.* Uganda, and *S.* Cerro. More than half of the strains were resistant to at least one antimicrobial and half of them to at least three antimicrobials. Resistance was more frequently observed to streptomycin, tetracycline, and sulfisoxazole. Interestingly, most *S.* Typhimurim and all *S.* Dublin and *S.* Uganda strains were resistant to four or more antimicrobials, while most *S.* Infantis were resistant to only one antimicrobial. A total of 22 PFGE profiles were produced from 29 different isolates; multiple PFGE profiles were found in isolates originated from a same pork sample in three occasions. In contrast, only single PFGE profiles were identified among isolates recovered from each positive beef sample.

Significance: Our findings indicate that *Salmonella* was present in retail beef and pork products and represented multiple serotypes exhibiting various antimicrobial resistances.

P2-140 Superior Resuscitative Nature of Enrichment Broth for the Detection of Pathogenic Serotypes of *Escherichia coli* (namely O157)

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Introduction: Cattle have been identified as a major reservoir of pathogenic serotypes of *Escherichia coli* (specifically O157) and foods of bovine origin, including beef and dairy products, as well as plant products have been implicated in numerous outbreaks of infection worldwide. Reliable enrichment and detection systems are necessary to monitor foodstuffs (including rinse waters) for these pathogens to ensure that safe food is being supplied to the consumer.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® *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, dairy products, and plant products.

Methods: A method comparison study was conducted to determine accuracy, specificity, sensitivity and relative detection level. One hundred ninety-eight (198) samples were analyzed by both methods. Selectivity was evaluated by testing fifty (50) target microorganisms and thirty (30) non-target microorganisms.

Results: The test system demonstrated 85% relative accuracy. The relative sensitivity of the method was 85% and 92% for 8 and 24 hours respectively. The relative specificity was 85% and 74% for 8 and 24 hours, respectively. The relative level of detection of the test method varies from 0.6 to 1.0 CFU/25 g while the limit of detection of the reference method varies from 0.5 to 0.9 CFU/25g. 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 hours with the test system. Results obtained after 24 hours of incubation of the test system enrichment broth show superior results as compared to the reference method. 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.

P2-141 Spontaneous Excisions within the Sp11-Sp12 Prophage Region of *Escherichia coli* O157:H7 Sakai

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Developing Scientist Competitor

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC), especially those of serotype O157:H7, are foodborne pathogens that can cause serious diseases including hemorrhagic colitis and hemolytic uremic syndrome. Acquisition of many EHEC virulence genes including those encoding Shiga toxins and type III secretion system effector proteins has largely been driven by bacteriophage-mediated horizontal gene transfer. Meanwhile, curing of prophage has also been observed both in vitro and in vivo, which can result in strains with different pulsed field gel electrophoresis (PFGE) patterns during epidemiological investigations.

Purpose: Previous work in our laboratory suggested that the genome region encoding two tandemly integrated prophage, designated Sp11 and Sp12, undergoes spontaneous deletion(s). In this study we chose to further characterize these deletions to determine whether they may impact molecular subtyping results.

Methods: The counter-selectable marker *sacB* was integrated into Sp11 to quantify the frequency of prophage curing. The limits of deleted regions in each mutant were defined by PCR.

Results: Sucrose-resistant colonies (indicating loss of *sacB*) were observed at a frequency between $6.23x10^{-4}$ and $1.30x10^{-3}$. Out of 20 colonies screened, only 1 had a precise excision of Sp11. While we were unable to define the exact limits of the deletions in other colonies, we did observe 4 other deletion events that included both Sp11 and Sp12, of which the minimal deletion sizes ranged from 35 to 45 kb. Sequencing of the phage released from another clinical isolate of E. coli O157:H7 suggested that these regions become incorporated into mature phage particles.

Significance: This study identified a region of instability within the genome of E. coli O157:H7. Characterization of additional regions and determining the effects deletions have on PFGE patterns will help improve the accuracy of this molecular subtyping method.

P2-142 Performance of a Molecular Detection System for the Detection of *Salmonella* and *Escherichia coli* O157 in Food and Carcass Samples

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Poster

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Introduction: Testing for *Salmonella* and *E. coli* O157:H7 is a critical component of food safety programs as contamination by these pathogens can result in significant adverse health conditions and economic loss. The 3M™ Molecular Detection Assay Salmonella and the 3M™ Molecular Detection Assay *E. coli* O157 (including H7) were developed for the rapid and specific detection of these organisms after enrichment. The assay uses a combination of isothermal amplification of unique DNA target sequences and bioluminescence to detect the amplified sequences.

Purpose: To evaluate the performance of detection methods using a variety of foods and poultry and beef carcasses.

Methods: *Salmonella* and *E. coli* O157 were used to artificially contaminate > 95 food samples with 5-13 CFU of target pathogen per sample size. Additional > 140 samples beef and poultry carcasses were also tested. Two test periods were conducted: 1) one sample was enriched blank and one was artificially contaminated with ~10 CFU of the target organism and 2) samples were enriched blank to evaluate for native contamination. Enrichments were tested using detection molecular assays and selective and differential agar &/or quantitative PCR.

Results: Compared to agar or qPCR, accuracy, specificity and sensitivity were: 97.6%, 100%, and 93.9% for the molecular detection assay *Salmonella*, and 95%, 100%, and 92% for the 3M Molecular Detection Assay *E. coli* O157 (including H7).

Significance: The methods demonstrated comparable results to the other methods for the rapid, automated detection of these organisms.

P2-143 Improving Post-harvest Safety and Shelf-life of Whole Mangos

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Introduction: *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* and *Shigella flexneri* infections have been associated with the consumption of a number of fruits and vegetables including mangos.

Purpose: The aims of this investigation were to: a) study the effect of x-ray treatments in reducing *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* on whole mangos and b) study the effect of x-ray treatments on microflora counts (mesophilic counts, psychrotrophic counts and yeast and mold counts) of whole mangos during storage at ambient temperature 22°C for 30 days.

Methods: A mixture of three strains of each test organism was spot inoculated (100 µl), separately, onto the surface (5 cm^2) of whole mangos (approximately 8-9 log CFU ml^{-1}), air dried (30 min), and then treated with different doses of X-ray (0, 0.1, 0.5, and 1.0 kGy) at 22°C and 55% relative humidity at 22°C and 55–60% relative humidity. Surviving bacterial populations on whole mango surfaces were evaluated using a nonselective medium (tryptic soy agar) with a selective medium overlay for each bacterium; *E. coli* O157:H7 (CT-SMAC agar), *L. monocytogenes* (MOA), and *S. enterica* and *S. flexneri* (XLD).

Results: Approximately 2.2, 0.8, 4.1 and 2.0 log CFU reduction of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* were achieved by treatment with 0.1 kGy X-ray, respectively. Furthermore, the populations of *E. coli*O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* were reduced to less than the detectable limit (2 log CFU/g) by treatment with 1.5 X-ray. Treatment with 1.5 kGy X-ray significantly reduced the initial inherent microflora on green peppers and inherent levels were significantly ($P < 0.05$) lower than the control sample throughout storage at 22°C for 30 days.

Significance: These results showed that treatment with X-ray significantly reduced selected pathogens and inherent microorganisms on whole mangos, which could be a good alternative to other technologies for fruits industry.

P2-144 Effect of X-Ray Treatments on the Safety and Shelf-life of Parsley Leaves

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Introduction: The foodborne pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* and *Shigella flexneri* are ubiquitous bacteria widely distributed in agricultural environments and products including parsley.

Purpose: The objectives of this investigation were to: a) study the reduction of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* on parsley by X-ray and b) study the effect of X-ray treatments on microflora counts (mesophilic counts, psychrotrophic counts and yeast and mold counts) of untreated and treated parsley during storage at 5°C for 30 days.

Methods: Dip-inoculated method was used to inoculate parsley leaves, separately, with a mixture of three strains of each tested organism (approximately 8–9 log ml^{-1}), air-dried, and followed by treatment with different doses of X-ray (0, 0.1, 0.5, 1.0, and 1.5 kGy) at 22°C and 55–60% relative humidity. Surviving bacterial populations on parsley leaves were evaluated using a nonselective medium (tryptic soy agar) with a selective medium overlay for each bacterium; *E. coli* O157:H7 (CT-SMAC agar), *L. monocytogenes* (MOA), and *S. enterica* and *S. flexneri* (XLD).

Results: Approximately 5.8, 3.1, 5.7 and 5.8 log CFU reduction of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* were achieved by treatment with 1.0 kGy X-ray, respectively. Furthermore, the populations of *E. coli*O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* were reduced to less than the detectable limit (2 log CFU/g) by treatment with 1.5 X-ray. Treatment with 1.5 kGy X-ray significantly reduced the initial inherent microflora on green peppers and inherent levels were significantly ($p < 0.05$) lower than the control sample throughout refrigerated storage for 30 days.

Significance: The results of investigation indicated that X-ray is an effective technology to eliminate *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *Shigella flexneri* and extend the shelf life of parsley leaves.

Poster

P2-145 Growth Characteristics of *Listeria monocytogenes* Strains Serotype 1/2a and 4b Isolated from Food and Clinical Samples Submitted to Different Conditions

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Developing Scientist Competitor

Introduction: Control of *Listeria monocytogenes* in food processing plants is a difficult matter due to its ability to form biofilms and to adapt to adverse environmental conditions. Survival at high concentrations of sodium chloride and growth at refrigeration temperatures are two other important characteristics of *L. monocytogenes* isolates.

Purpose: To compare the growth characteristics of the most important *L. monocytogenes* serotypes from different origins submitted to stress conditions and different temperatures.

Methods: Twenty-two *L. monocytogenes* isolates−12 from clinical cases (eight serotype 4b and four serotype 1/2a) and 10 isolates from food (six serotype 4b and four serotype 1/2a) and one *L. monocytogenes* Scott A strain and one *Listeria innocua*−were analyzed for the ability to grow in brain heart infusion (BHI) broth with 11% NaCl at 4 °C, 10 °C and 25 °C for 73, 42 and 15 days, respectively.

Results: The majority of *L. monocytogenes* strains, regardless of their origin, showed ability to remain viable or even grow at 4 °C and in high osmotic conditions usually applied to control pathogens in the food industry. At 10 °C most strains could adapt and grow, however there was no

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statistical difference ($P > 0.05$) for parameters such as lag phase duration, maximum growth rate and maximum cell density. At 25 °C all strains were able to grow and increase their population up to 5 log CFU/ml. Statistical analysis for this condition showed significant differences ($P < 0.05$), with clinical strains showing longer lag phase and lower maximum cell density compared to food strains.

Significance: The global tendency in decreasing NaCl concentration in processed foods, for health reasons, may facilitate *L. monocytogenes* survival and growth in these products. Therefore, food companies will have to look for additional growth barriers to assure product safety.

P2-146 Prior Exposure to High Fat Content and Low Water Activity Improves the Survival of *Salmonella enterica* Tennessee in a Simulated Gastrointestinal System

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Developing Scientist Competitor

Introduction: A surge of documented foodborne outbreaks caused by *Salmonella enterica* serovars have been linked to foods with high fat content and low water activities (a_w). The low a_w (0.3) of peanut butter limits the growth of*Salmonella* in the product; however, illnesses are reported from peanut butter contaminated with very small doses, suggesting the infectious dose of *Salmonella* is much lower when delivered in peanut butter.

Purpose: The purpose of our study was to quantify the survival of *Salmonella* exposed to peanut butter with different fat content and water activities after prolonged storage and after passage through a simulated gastrointestinal system.

Methods: *S. enterica* Tennessee (the strain from the 2006-2007 peanut butter outbreak) was inoculated into four peanut butter treatments (high fat, high a_w; high fat, low a_w; low fat, high a_w; low fat, low a_w) and then challenged with the simulated conditions of the gastrointestinal tract.

Results: *Salmonella* survival after *in vitro* digestion was influenced primarily by the fat content of the peanut butter. High fat/high a_w and high fat/low a_w peanut butter had a 2.4-log and 2.0-log increases in numbers, respectively, while low fat with both high and low a_w peanut butter peanut butter had 1.2-log reductions in cell count ($P < 0.02$).

Significance: Further studies should investigate the high fat effects on long-term storage and heat treatments in conjunction with *in vitro* digestion models. The importance of prior stress response on survival and virulence should be considered for risk analysis and in developing regulations.

P2-147 Application of PCR/MS Methodology to the Detection of Enteric Bacterial Pathogens in Food Samples

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Introduction: PCR combined with mass spectrometry can be used as a rapid method for detecting bacterial pathogens in foods. The foodborne pathogen assay under investigation allows for identification of *Salmonella*, *Shigella*, or pathogenic *E. coli* at the serovar, species or serogroup level, respectively, within a few days of sample collection.

Purpose: The objective of this study was to test the ability of a PCR/MS assay to detect and identify foodborne bacteria in spiked and naturally contaminated food products.

Methods: Both spiked food samples and foods collected during the regulatory process were analyzed with the assay. After pre-enrichment and selective enrichment incubations, DNA was extracted directly from the broth and analyzed on the assay. Limits of detection and sensitivity in the presence and absence of background microflora were determined for different pathogens.

Results: *Salmonella* was detected in 9 of 10 culture-positive selective enrichment broths from naturally contaminated samples. The serovar identified by serology was a match to PCR/MS results in 8 of 10 samples. Analysis of pre-enrichment broth was less successful in the analysis of both naturally contaminated and spiked foods where the correct serovar was identified in 29 of 48 spiked samples (60%). Serovar-level identification of *Salmonella* was performed with as little as 80 pg of DNA (pure sample).

Significance: The development of rapid bacterial pathogen detection and identification assays will improve response times to public health events. This work indicates that PCR/MS could be a vital tool for quickly assessing possible vehicles in foodborne pathogen outbreaks.

P2-148 Monitoring of O26, O103, O111, O145 and O157 Shiga Toxin-producing *Escherichia coli* in Slaughtered Cattle by a Real-Time PCR-based System

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Introduction: Most cases of severe STEC infections in humans are attributed to STEC O157:H7, but the importance of non-O157 STEC is increasingly recognized. For monitoring of STEC in cattle, the European Food Safety Authority proposed to screen for STEC O26, O103, O111, O145 and O157, the so-called top-five serogroups.

Purpose: The aims of this study were (i) to screen bovine samples by real-time PCR for Shiga toxin genes and the top-five serogroups, and (ii) to isolate strains from selected PCR-positive samples.

Methods: From 573 slaughtered cattle (aged 3-24 months) fecal samples were collected in seven abattoirs. After enrichment (mTSB with novobiocin), samples were examined by real-time PCR for *stx* and if positive for the top-five STEC serogroups. From the collective of O26, O145 and O157 PCR-positive samples, strain isolation was attempted by serogroup-specific colony dot-blot hybridization after immunomagnetic separation. Isolated strains were examined for *stx*1, *stx*2 and*eae* (intimin).

Results: Of 563 samples with evaluable results, 74.1% tested positive for *stx*. Amongst them, serogroups O145, O103, O26, O157 and O111 were detected in 41.9%, 25.9%, 23.9%, 7.8% and 0.8%, respectively. From 95 O26, 166 O145 and 30 O157 PCR-positive samples, 17 O26, 28 O145 and 12 O157 strains were isolated. The 17 O26 strains were *eae*-positive but only nine strains harbored *stx* (eight possessing *stx*1 and one *stx*2). Of the 28 O145 strains, ten were *eae*-positive including four harboring *stx*1 or *stx*2, whereas 18 were negative for *stx* and *eae*. Five of the 12 O157 strains harbored *stx*2 and *eae*, did not ferment sorbitol, and were identified as STEC O157:H7/H-. The other seven O157 strains were negative for*stx* and *eae* or positive only for *eae*.

Significance: Shiga toxin genes were frequently found in young Swiss cattle at slaughter and the top-five STEC serogroups, especially O26, O103 and O145, were also detected amongst the *stx*-positive samples. However, success rates for strain isolation were low and only few of the isolated strains showed a virulence pattern of human pathogenic STEC.

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P2-149 Prevalence of *Escherichia coli* O157:H7 in Small-scale Cow/Calf Operations

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Introduction: Foodborne illnesses have been associated with *Escherichia coli* O157:H7 since this organism was first reported in 1982. Several studies have indicated that cattle herds worldwide are the primary reservoirs of *E. coli* O157:H7. The main sources of *E. coli* O157:H7 in feedlots are feces, feed bunks, water troughs and incoming water supplies. There is limited information available on the prevalence of *E. coli* O157:H7 at small-scale cow/calf operations.

Purpose: The purpose of the study was to establish the prevalence of *E. coli* O157:H7 on small-scale cow/calf operations in the state of Louisiana.

Methods: The study was done between June and December 2011. Samples collected and tested were: fresh fecal matter on the ground, water from troughs and swabs from troughs, salt and hay bunks. For the isolation of the *E. coli*O157:H7, samples were enriched in TSB, followed by an immunoseparation and the final step was plating on CT-SMAC and CHROMagar®. RT-PCR was used to identify the strain by using the FDA's BAM methodology and the genes were stx_1, stx_2,*eae*, and $fliC_{h7}$.

Results: A total of 420 fecal samples, 168 water samples and 56 swabs were taken from 27 farms. From which, 9%, 7% and 2% were positive, respectively. This shows that although fecal matter has a higher prevalence, water troughs are a source of *E. coli* O157:H7 as well. On the contrary, the low percentage of positives for the swabs indicated that surfaces have a minor role in the prevalence of this pathogen in cattle operations.

Significance: These findings can be used to help establishing Best Management Practices and pre-harvest food safety for small-scale cow/calf operations.

P2-150 Growth and Survival of *Salmonella* in Ground Black Pepper

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Introduction: In 2010, a large outbreak of salmonellosis was attributed to the consumption of contaminated black pepper. Black pepper and white pepper have previously been linked to foodborne disease outbreaks. The survival and growth of *Salmonella* in black pepper are not well understood. A better understanding of *Salmonella* in dry products will enhance risk assessments for these products.

Purpose: To determine if *Salmonella* can grow and establish stable populations on ground black pepper and to determine the minimum water activity for such growth to occur.

Methods: Unsterilized and sterilized commercial ground black pepper at different water activity levels was inoculated with a three serovar cocktail of *Salmonella* at a starting level of approximately 4-5 log CFU/g and then incubated at 25 and 37°C. Immediately after inoculation and at appropriate intervals over a 48-hour period, triplicate samples were plated on both trypticase soy agar with yeast extract (TSAYE) and xylose lysine deoxycholate agar (XLD) for microbial enumeration.

Results: At a water activity of 0.9916-0.9884, the generation time at 37 and 25°C for *Salmonella* was 0.54 and 1.22 h, respectively, with lag times of 4 to 6 h. Reducing the water activity to 0.9756-0.9739 increased the generation time to 3.96 and 4.93 h at 37 and 25°C, with lag times of 18-24 h. Growth was not observed at water activities below 0.97. The presence of background microflora appeared to enhance growth and survival of *Salmonella*.

Significance: *Salmonella* can grow on black pepper at an alarmingly fast rate when the water activity is permissive. Black pepper having permissive water activity may not appear noticeably wet. Native background microflora on black pepper may enhance *Salmonella* survival.

P2-151 Variation in Confirmation Rates of Samples Screen-positive for *Escherichia coli* O157 in Beef Trim by Using PCR to Screen for Virulence-associated and Serotype-specific Targets

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Introduction: Confirming the presence of *Escherichia coli* O157 in beef trim samples requires tests subsequent to screening. Because of time factors, beef processors may rely on screening methods to flag potentially positive samples and make decisions about product lot disposition. While screening methods must be sensitive, too many "false-positive" screens can lead to product disposition that is economically unfavorable for the producer.

Purpose: In this study, we determine the confirmation rate of screen-positive beef trim samples for a method to detect virulence-associated and serotype-specific genes that are found in *E. coli* O157 and to determine if there are factors such as processing plant, month and sample lean content that affect this rate.

Methods: From 15 different processing plants that reported O157 confirmation test results during calendar year 2010, 460,137 trim samples (375 g, 60 surface pieces) were enriched ≥ 9 h and then screened by a multiplex PCR targeting the *rfb*, *stx* and *eae* genes and a lateral flow (LF) immunological test for the O157 antigen. Samples which were rfb^+ and/or LF^+, eae^+ and stx^+ (stx_1^+ and/or stx_2^+) were designated as O157 IR (Initially Reactive). Confirmation tests included immunomagnetic separation using anti-O157 antigen beads and PCR assays for *rfb*, *eae*, H7, *γint*, subtilase, and *stx*.

Results: Overall, 15.5% of the O157 IR samples (which signals come from mixed enrichment cultures) were confirmed positive for *E. coli* O157 by subsequent molecular tests. During the year, confirmation rates ranged from 5.9-25.6% and tended to be higher from late mid-spring through fall. For PCR signals, results ranged from 7.3-20.6% with stx_2^- the lowest and stx_1^- the highest. Confirmation rates for processing plants ranged from 3.2-72.13%. Samples 50-69 and 70-89 percent lean had confirmation rates of 19.9 and 12.%, respectively.

Significance: Overall, 15% of screen-positive samples remained positive after confirmatory testing. Confirmation rates varied by month, by plant, individual gene tests and less so by day of the week. Sampling plans might be designed to take these variations into account, especially during the summer and fall. Variations between plants could be used to examine their processing controls.

P2-152 Survivability of MNV and MS2 on Either Wood or Stainless Steel Surfaces at Various Temperature and Relative Humidity

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Introduction: Norovirus (NoV) is one of the common causes of non-bacterial acute gastroenteritis. They are known to persist on food-contact surfaces for prolonged periods, which lead to cross contamination of foods and foodborne diseases. Hence, we characterized the survival

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of murine norovirus (MNV) as a surrogate of human norovirus (HuNoV) and bacteriophage MS2 on wood or stainless steel surface under temperatures (4, 15, 25, 32, and 40°C) and relative humidity (30, 50, and 70%).

Purpose: To characterize the inactivation curves of MNV and MS2 under different environment conditions.

Methods: About 10^8 PFU/ml was inoculated, sampled at predetermined time over 30 days, and analyzed by both plaque and real-time RT-PCR assays. The Weibull model and D-value were used to describe inactivation in the environmental conditions.

Results: Both MNV and MS2 survived better on wood surface than stainless steel. As both temperature and RH increased, both MS2 and MNV were inactivated more rapidly. For example, more than a 4-log reduction of MNV occurred on stainless steel surface at 40 and 70% RH within 9 hours. However, only about a 2-log reduction of MNV occurred on wood surface at 25 and 30% RH over 30 days. Regardless of tested conditions, viral RNA was persistent without any significant reduction.

Significance: Our results demonstrated that both surface characteristics and environments could influence viral survivability.

P2-153 Expression of the Virulence Gene *hilA* in *Salmonella enterica* is Suppressed by *Lactobacillus casei* Secondary Metabolites

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Developing Scientist Competitor

Introduction: *Salmonella* spp. are the leading cause of foodborne illness among humans. The intestinal track of poultry can become colonized with *Salmonella* without causing harm or illness to the bird, which may lead to the potential contamination of poultry products during processing. Thus, pre-harvest intervention measures that aim to reduce colonization have the potential to reduce raw product contamination. *Lactobacillus* also colonizes the intestinal tract of poultry and has been utilized as a probiotic culture to eliminate *Salmonella* from the intestines of poultry. However, exactly how *Lactobacillus* eliminates *Salmonella* is not completely understood, but has been partially explained by the production of antimicrobial substances and by competing for niches and nutrients. We hypothesized that secondary metabolites produced by *Lactobacillus* may interfere with the expression of genes involved in colonization and virulence in *Salmonella*.

Purpose: Thus, the purpose of these experiments was to measure any changes in gene expression of *Salmonella* in response to exposure to secondary metabolites of *Lactobacillus casei*.

Methods: *Salmonella enterica* serovars Typhimurium (DT104) and Enteritidis (wild-type) and *Lactobacillus casei* (ATCC 334 and 393) were cultured in Man-Rogosa-Sharp broth (MRS). *Lactobacillus cultures* then were filter sterilized to produce sterile spent MRS medium and the pH was adjusted to either 6.2 or 5.5. *Salmonella* cultures were centrifuged and resuspended in either the spent or sterile, fresh MRS with a pH adjusted to 6.2 or 5.5. Total RNA then was extracted from these resuspended cultures at specific time points (0, 2, 4 and 24 hours) and the expression of *hilA* was evaluated using reverse transcriptase real-time PCR (RT-PCR).

Results: The *hilA* gene was upregulated in *Salmonella* when cultures were suspended in sterile, fresh medium with a slightly acidic pH. However, upregulation of the expression of *hilA* was suppressed in *Salmonella* cultures that were suspended in the sterile spent medium.

Significance: From these experiments, it appears that secondary metabolites produced by *Lactobacillus casei* suppress the upregulation of the virulence regulator *hilA*. Further work will be conducted to determine what metabolites are specifically involved in the suppression of the *hilA* gene. In addition, a global view of all genes under these conditions will be conducted utilizing a microarray approach to understand the full impact of the *Lactobacillus* secondary metabolites on*Salmonella* gene expression.

P2-154 Comparative Expression Analysis of Two Thermostable Nuclease Genes in *Staphylococcus aureus*

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Introduction: Thermonuclease is known as a specific virulence factor in *Staphylococcus aureus*. A remarkable tolerance to prolonged heating and storage is exhibited by staphylococcal thermonuclease in foods and broth, and its presence is closely related with the occurrence of enterotoxins in food poisoning outbreaks. Previous studies have revealed the existence of two functional thermostable nucleases encoded by two different genes (*nuc1* and *nuc2*) in *S. aureus*.

Purpose: To understand how these two thermonuclease genes are regulated in different strains of *S. aureus*, expression characteristics of both two genes are required.

Methods: Comparative mRNA analysis of *nuc1* and *nuc2* was carried out by Taqman-based real-time PCR. Toluidine blue DNA agar and spectrophotometric assay were used for thermonuclease activity test.

Results: Distinct expression patterns were observed at different growth stages, and expression was under the control of the *sae* regulatory system in strain RN4220. Maximum level of *nuc1* transcripts was at the post-exponential growth phase, and expression was down-regulated 24 fold at late-exponential phase in a *sae* mutant. In contrast, *nuc2* transcript levels declined after the early exponential phase, and it was 1.4 fold up-regulated in the *sae* mutant. Furthermore, unlike the expression of *nuc1* that varied in three different *S. aureus* clinical strains, the transcription of *nuc2* remained relatively constant. The *nuc1* transcription level correlated well with thermonuclease activity results, which suggest that *nuc1* plays a primary role in thermonuclease activity in *S.aureus*.

Significance: This information will be useful for understanding thermonuclease gene function and alterations of regulation for pathogenesis and food poisoning of *S. aureus*.

P2-155 Evaluation of a Next-day Method for Detection of *Listeria monocytogenes* in Food

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Introduction: The VIDAS® *L. monocytogenes* Xpress (LMX) is a newly developed, automated assay using the ELFA (Enzyme Linked Fluorescent Assay) principle and designed for use in the VIDAS® instrument.

Purpose: A study was conducted to validate the LMX for detection of *Listeria monocytogenes* in food. This study was conducted as part of the AOAC Research Institute approval process.

Methods: In the LMX method, samples are enriched for 26-30 h in LMX broth then an aliquot of the broth is transferred to the test strip, heat-treated for 5 min and cooled before testing. The assay gives a result for presence of *L. monocytogenes* within 80 minutes. Enriched samples were confirmed by streaking to Oxford agar, Chrom ID Ottaviani Agosti agar (OAA) and Chrom ID *L. mono* agar. Biochemical confirmation was performed by Vitek1 (AOAC method 991.13). The study included 6 foods (processed cheese, vanilla ice cream, frozen spinach, peanut butter, cooked shrimp and smoked cod). Twenty 25-g samples inoculated at a low level (target 0.2-2 CFU/25 g) and 5 x 25 g uninoculated samples were

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tested using the LMX assay and the appropriate reference method (AOAC 993.12 for cheese and ice cream) and the FDA BAM method for remaining foods.

Results: Results for the 6 foods showed that there was no significant difference between the LMX method and the reference methods for detection of *L. monocytogenes* using an unpaired chi-square test at 5% level. Confirmation using traditional and chromogenic agars gave equivalent results.

Significance: The LMX assay provides a presumptive result for the presence of *L.monocytogenes*, within 28 hours. The assay can detect the presence of the pathogenic species, *L. monocytogenes*, faster than traditional methods and other immunoassays.

P2-156 A Comparative Evaluation of the GeneDisc® Plate *Listeria* Duo for the Detection of *Listeria monocytogenes* and *Listeria* species in a Variety of Foods and Environmental Surfaces

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Introduction: The GeneDisc® Plate *Listeria* DUO utilizes qPCR reactions to amplify and detect specific DNA sequences for simultaneous detection of 6 species of *Listeria*. After enrichment in demi-Fraser broth for 24 hours at 37 °C, sample DNA is extracted, combined with Master Mix reagent, sealed and analyzed using the Cycler. Up to 8 plates containing 6 or 12 samples can be analyzed at one time. Each plate contains positive and negative control wells to validate sample analysis

Purpose: The purpose of this internal validation was to evaluate the robustness, product consistency, stability, instrument variation and inclusivity/exclusivity of the candidate method and compare to the FDA/BAM and USDA/FSIS-MLG reference methods as part of the AOAC-RI™ PTM validation.

Methods: The method comparison was conducted on 10 food matrices and 2 environmental surfaces by the candidate method and the reference methods. Each matrix was inoculated with a different strain of both *Listeria monocytogenes*and *Listeria* species. 20 replicates were analyzed at one inoculum level: 0.2-2 CFU/25g. Five control replicates were analyzed at 0 CFU/25g. Inclusivity/exclusivity, robustness, stability, product consistency and instrument variation were evaluated.

Results: A POD statistical analysis indicated no significant differences between the new method and the reference methods for *Listeria* species and *L. monocytogenes* for all 12 matrices. For inclusivity, 50 out of 50 strains of *L. monocytogenes*and 10 out of 10 strains each of *L. grayi, L. innocua, L. ivanovii, L. seeligeri*, and *L. welshimeri* were correctly identified. All 30 exclusivity organisms were correctly excluded. The robustness results indicated minor variations to testing parameters had no effect on final results. Stability results indicate performance was consistent over the 6 month shelf-life. Product consistency and instrument variation results demonstrated consistency between lots of assay and instruments.

Significance: This new method is a rapid, reliable alternative to the traditional method for simultaneous detection of *Listeria monocytogenes* and *Listeria* species.

P2-157 A Comparative Evaluation of the GeneDisc® Plate *Listeria* Identification Kit for the Identification of *Listeria* Species

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Introduction: The GeneDisc® Plate *Listeria* Identification Kit enables the identification and discrimination of 6 *Listeria* species from isolated colonies utilizing Real-Time PCR technology. Sample DNA is extracted from isolated colonies on one of 6 different agars, combined with Master Mix reagent, sealed and analyzed using the Cycler. Up to 8 plates containing 6 or 12 samples can be analyzed at one time with each plate containing positive and negative control wells to validate sample analysis.

Purpose: The purpose of this internal validation was to evaluate the ruggedness, product consistency, stability, instrument variation and inclusivity/exclusivity of the candidate method and compare it to the FDA/BAM and USDA/FSIS-MLG biochemical confirmation process as part of the AOAC-RI™ PTM validation.

Methods: A method comparison of 10 food matrices and 2 environmental surfaces was conducted using the GeneDisc® Plate *Listeria* ID and the FDA/BAM or USDA/FSIS-MLG reference methods. For confirmation of samples by the ID method, each matrix was streaked to 6 different agars (MOX, OX OAA, Palcam, Rapid'L.mono™ and Nutrient Agar). Typical *Listeria* colonies from each agar were picked for identification by the candidate method. Inclusivity/exclusivity, stability, product consistency and instrument variation parameters were evaluated.

Results: For the method comparison, the candidate method correctly identified 722 out of 727 isolates (99.3%). For inclusivity, 837 out of 840 isolates (99.6%) were correctly identified. All 30 exclusivity organisms were correctly excluded. The robustness results indicated minor variations to testing parameters had no effect on final results. Stability results indicate performance was consistent over the 6 month shelf life. Product consistency and instrument variation results demonstrate consistency between lots of assay and instruments.

Significance: The candidate method demonstrated high specificity in the identification and discrimination of *Listeria* species.

P2-158 Comparison of Different Preenrichment Broths: Preenrichment Broth Ratios and Surface Disinfection for the Detection of *Salmonella* Enteritidis in Shell Eggs

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Introduction: The large multistate *Salmonella* Enteritidis (SE) outbreak associated with shell eggs in 2010 underlines the importance of monitoring SE in shell eggs and egg products. The current FDA's Bacteriological Analytical Manual (BAM) method for detecting SE in eggs is a lengthy procedure, which takes two weeks to complete.

Purpose: The objective of this project is to provide the scientific bases for how to improve the conventional detection method for SE in shell eggs in FDA's BAM.

Methods: Two SE isolates (2 phage types) were used for comparison of five different preenrichment broths, and evaluation of egg:preenrichment broth ratios for the detection of SE in shell eggs. The effect of surface disinfection on the detection of SE in shell eggs was also investigated. The experiment was conducted in three replications.

Results: The results indicated that tryptic soy broth (TSB) was similar to TSB plus ferrous sulfate ($FeSO_4$); but significantly ($\alpha = 0.05$) better than nutrient broth, universal pre-enrichment broth, and buffered peptone water when used for preenrichment of SE in eggs. Egg:broth ratios at 1:9 and 1:2 produced significantly ($\alpha = 0.05$) higher SE populations after preenrichment with TSB with inoculum levels at 4 CFU SE/100 g

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eggs and 40 CFU SE/1,000 g eggs than the ratio at 1:1. SE populations in TSB preenrichment cultures of shell eggs surface-disinfected with 70% alcohol:iodine/potassium iodide solution and untreated control were 9.11 ± 0.11 and 9.18 ± 0.05 log CFU/ml for SE 13-2, respectively; 9.20 ± 0.04 and 9.16 ± 0.05 log CFU/ml for SE CDC_2010K_1543, respectively.

Significance: In conclusion, preenrichment with TSB was as effective as with TSB plus $FeSO_4$. Surface disinfection of eggs had no effect on SE populations in the preenrichment culture. These discoveries can substantially reduce the workload and shorten the detection time for SE in shell eggs and eventually improve the current FDA's BAM method for detection of SE in shell eggs.

P2-159 Single Laboratory Validation of a *Vibrio* Assay for Identification of *Vibrio* Isolates

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Introduction: *Vibrio cholerae* (Vc), *V. parahaemolyticus* (Vp), and *V. vulnificus* (Vv) are well-documented human pathogens associated with seafood consumption. The FDA BAM recommends enrichment in alkaline peptone water (APW) followed by colony isolation on selective/differential agar. Biochemical testing or conventional PCR procedures are recommended for identification of suspect *Vibrio* isolates**.**

Purpose: This study compares the BAX *Vibrio* assay to the BAM procedure for identification of *Vibrio* isolates.

Methods: *Vibrio* cultures were grown in APW overnight at 35 °C, streaked onto selective agars and confirmed biochemically as Vc, Vv, or Vp using API20E. For the BAM PCR confirmation, a crude lysate was prepared by boiling the overnight APW enrichment and 2 µl was used as template for detection of Vv (519 bp species-specific fragment of *vvh*) and Vp assay (450 bp species-specific fragment of *tlh*). PCR products were visualized by gel electrophoresis as described in the BAM. For the BAX *Vibrio* assay, the manufacture instructions were followed for multiplex qPCR detection of Vc, Vv, and Vp as described by the manufacturer.

Results: The BAX *Vibrio* assay correctly identified 50/52 Vc isolates while API20E identified all 52. For Vv*,* 51/51 isolates were positive by the BAX *Vibrio* assay, 45/51 by API20E and 50/51 by the FDA BAM PCR. Both the BAX *Vibrio* assay and API20E identified all 55 Vp isolates correctly and the BAM PCR confirmed 54/55. Of the 30 near-neighbor *Vibrio* isolates, as identified by API20E, none were detected by the BAX *Vibrio* assay.

Significance: The results presented here demonstrate the BAX *Vibrio* assay is a reliable and rapid alternative to the BAM methods for identification of Vc, Vv, and Vp isolates.

P2-160 Development of DNA Microarray Chip Containing Non-sequenced Genomic DNA Fragments for the Detection of *Listeria monocytogenes* in Milk

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Developing Scientist Competitor

Introduction: To maintain microbiological safety of foods, development of a rapid and sensitive detection method is required for the detection of foodborne pathogens. DNA microarray technology can meet these ideal demands.

Purpose: The objectives of this research are to develop a DNA microarray containing genomic DNA fragments of *Listeria monocytogenes*, to verify its diagnostic ability using a laboratory medium, and to apply the microarray to detect *L. monocytogenes* in milk.

Methods: Sixty genomic DNA fragments of *L. monocytogenes* digested using several pairs of restriction enzymes were randomly selected and affixed to a slide glass to fabricate the DNA microarray. The diagnostic ability of the microarray was verified using seven strains of *L. monocytogenes*, seven strains of other *Listeria* spp., and thirteen strains of foodborne pathogens in different genera. Finally, the diagnostic ability of the DNA microarray in milk was confirmed and the sample preparation procedure was established.

Results: DNA microarray was able to detect *L. monocytogenes* and distinguish it from other *Listeria* spp. and other foodborne pathogens in a laboratory medium. The detection limits of the microarray were approximately 8 log CFU/ml when other types of bacterial strains (*B. cereus*, *S.* Montevideo, *Bacillus* spp., *P. aeruginosa*) were present in high number in milk. For the enrichment at 37 °C, UVMB was better than TSB or BHIB in increasing the population of *L. monocytogenes*. When milk containing *L. monocytogenes* (2.2 log CFU/ml) and other five types of bacterial strains (> 2.0 log CFU/ml each) was enriched in UVMB at 37 °C for 24 h, the populations of *L. monocytogenes* increased to 7.7 log CFU/ml. When genomic DNAs extracted from these enriched suspensions were hybridized on the microarrays, *L. monocytogenes* were successfully detected.

Significance: The microarray containing non sequenced genomic DNA fragments was able to detect *L. monocytogenes* in milk without any interference of other microbes.

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P2-161 Effects of Gamma Radiation on Shiga Toxin-producing *Escherichia coli* Inoculated in Spinach (*Tetragonia expansa*) and on Sensory Characteristics

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Introduction: Vegetables, including spinach, have been associated with many foodborne outbreaks. Gamma radiation can be used to reduce the population of pathogenic bacteria on food or to increase the shelf life. However, depending on the dose used, it can cause undesirable changes in sensorial attributes of food.

Purpose: To evaluate the efficacy of irradiation on the reduction of Shiga toxin-producing *E. coli*, and the effect on the sensory characteristics of minimally-processed spinach.

Methods: Samples of spinach (*Tetragonia expansa*) were inoculated with a cocktail of three strains of Shiga toxin-producing *E. coli* O157:H7 (10^7–10^8 CFU/ml) and exposed to doses of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 kGy. Surviving bacterial populations were evaluated using a non-selective medium (Tryptic Soy Agar) with an overlay of Sorbitol MacConkey agar plus cefixime and potassium tellurite. Samples of non-spiked spinach exposed to 1.0 and 1.5 kGy and control sample, kept under refrigeration (4 °C) for up to 12 days were evaluated for appearance, flavor, smell and texture.

Results: The D_{10} values determined for Shiga toxin-producing *E. coli* was 0.17 kGy. All samples were accepted by the sensory panel with no statistically significant differences ($P < 0.05$) among the control and test samples.

Significance: The results showed that gamma radiation can be used for improving the quality of minimally-processed spinach without impairing its sensory characteristics.

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P2-162 Thermal Inactivation Kinetics of Murine Norovirus and Feline Calicivirus

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Introduction: Due to the recent rise in the number of foodborne norovirus outbreaks, novel preservation methods have increasingly focused on their control. Determination of required thermal treatment parameters to inactivate microorganisms is based on precise understanding of their inactivation kinetics. In the absence of human norovirus infectivity assays, cultivable surrogates such as feline calicivirus (FCV-F9) and murine norovirus (MNV-1) have been used to begin to understand thermal inactivation behavior.

Purpose: This study aimed to characterize the thermal inactivation kinetics of MNV-1 and FCV-F9 at 55, 65 and 72 °C for different treatment times (0-3 min).

Methods: Thermal inactivation was performed using the capillary tube method on the surrogate viruses at titers of 4.0×10^7 (MNV-1) and 5.8×10^8 (FCV-F9) plaque forming units (PFU)/ml, followed by standard plaque assays in duplicate. Each treatment was replicated thrice and data analysis was performed. Inactivation data were fitted to the Gompertz, logistic, Weibull and first-order models and compared statistically using regression coefficients (R^2), chi square (χ^2), and root mean square error (RMSE) values.

Results: Complete inactivation was achieved for both norovirus surrogates at 65 and 72 °C (> 6.68 log PFU/ml after 2 and 1 min, respectively); however, the total mean reduction at 55 °C was 0.83 log PFU/ml and 0.48 log PFU/ml for MNV-1 and FCV-F9 after 3 min, respectively. To determine thermal inactivation kinetics for the norovirus surrogates, all models were compared at each temperature. Results indicated that the model appropriateness was virus strain and temperature dependent.

Significance: Thus, use of multiple models is recommended and necessary for the precise determination of virus inactivation characteristics. The logistic model was found appropriate for both norovirus surrogates at 55 °C; however, at 65 and 72 °C the appropriate model was the Weibull model for MNV-1 and Gompertz model for FCV-F9. These results provide the parameters and data/thermal process calculations to accomplish thermal inactivation of norovirus surrogates.

P2-163 Pan-genomic Characterization of *Listeria monocytogenes* Strains Associated with the 2011 Cantaloupe Outbreak

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Introduction: *Listeria monocytogenes* is a Gram-positive foodborne pathogen responsible for listeriosis, an invasive bacterial infection in susceptible groups such as pregnant women, neonates and immuno-compromised adults. In September 2011, several listeriosis cases were first reported by the Colorado Department of Public Health and Environment (CDPHE). Follow-up investigation by the local, state and federal public health and regulatory agencies led to the discovery of a multistate listeriosis outbreak linked to the consumption of contaminated Rocky Ford cantaloupes. We have analyzed some environmental and food isolates implicated in this multi-state outbreak using a multiplex PCR and a microarray-based method to identify their serotypes and relatedness to each other and to other listeriosis outbreak strains.

Purpose: The objective for this study is to characterize genetic relatedness and determine the gene contents of the 2011 cantaloupe outbreak strains.

Methods: We have serotyped 27 *L. monocytogenes* strains associated with the 2011 cantaloupe outbreak using a combination of a slide-agglutination and a multiplex PCR assay. Seven strains from this outbreak and one unmatched strain from this group have further been analyzed using a microarray-based method (*Listeria* Gene-chip) to identify the genetic differences. The genomic DNAs isolated from these strains were hybridized onto the *Listeria* gene chip that was designed based on 24 *L. monocytogenes* publicly available genome sequences. The relatedness analysis was determined by comparing the gene content information of these cantaloupe outbreak strains against the database previously constructed from gene contents of 38 *L. monocytogenes* strains in our collection.

Results: The genotypic microarray analysis indicated that the cantaloupe outbreak strains are clustered in two different groups dividing three strains into the serotype 1/2a and five strains into serotype 1/2b, in agreement with PCR serotyping results. However, the cantaloupe outbreak strains are more closely related to each other than to the other strains in our database. The detailed analysis also revealed that the genetic contents of these two groups of strains are very distinct. Among the outbreak strains all of the PFGE cluster 2 belonged to the serotype 1/2b while strains in PFGE cluster 3 and 4 belonged to serotype 1/2a. One strain, LS667 of the PFGE cluster 4 pattern also belonged to serotype 1/2b, which was confirmed by our microarray-based relatedness analysis.

Significance: We show that genotyping microarray grouped the *L. monocytogenes* strains associated in the 2011 cantaloupe outbreaks in two distinct genomic clusters−one containing 1/2a strains and the other containing 1/2b strains. Cantaloupe outbreak-related 1/2a and 1/2b strains appeared to be genetically distinct from the previously known 1/2a and 1/2b outbreak strains. In addition, the microarray analysis and PCR serotyping revealed that strains of the PFGE cluster 4 belonging to both serotypes 1/2a and 1/2b.

P2-164 Comparison of Growth Kinetics for *Bacillus cereus* and *Bacillus cereus* Spore and *Staphylococcus aureus* in Blanched Wild Vegetables

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Introduction: There is growing demand in Korea for preprocessed wild vegetables as healthy and functional diets. Various wild vegetables at retail market or foodservice establishment are contaminated with *B. cereus* and *S. aureus* and are often sold at ambient temperature.

Purpose: The object of this study was to compare growth kinetics of *B. cereus* vegetative cell and spore and *S. aureus* in various blanched wild vegetables stored as a function of temperature.

Methods: We used lotus roots, doraji, burdock, gosari, and chwinamul as most popular wild vegetables(roots) in Korean diet. They were blanched in boiling water for 2 min. One hundred μl of vegetative cell and spore of *B. cereus* and *S. aureus* producing Enterotoxin A were inoculated into blanched wild vegetables (roots) at 10^3 CFU/ml, respectively and incubated at 13, 24 and 35 °C. Lag time (LT) and specific growth rate (SGR) of *B. cereus* and *S. aureus* were calculated from Gompertz equation at each temperature.

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Results: SGR of *B. cereus* spore was faster than that of *B. cereus* vegetative cell, regardless of kind of vegetables at 24 and 35 °C, while LT of *B. cereus* spore was longer than that of *B. cereus* vegetative cell, except for burdock. The SGR of*S. aureus* was faster than those of *B. cereus* at 24 and 35 °C, but no strain difference was observed at 13 °C. Compared to *B. cereus,* LT of *S. aureus* was shorter at 13 and 24 °C. *B. cereus* growth was not observed in gosari at 13 °C and in lotus roots at 13, 24 and 35 °C and *S. aureus* growth was observed in lotus roots at only 35 °C.

Significance: Proper temperature control during transportation and at retail market must be emphasized to control the growth of pathogens and spore germination. Since growth behavior of pathogens varies, more attention should be taken for storage temperature according to the kind of wild vegetables.

P2-165 Thermal Inactivation of *Staphylococcus aureus* in Ready-to-Heat Sauces

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Introduction: Although ready-to-heat (RTH) sauce outbreaks related to *Staphylococcus aureus* have occurred, the thermal resistance of *S. aureus* in RTH sauces has not been evaluated.

Purpose: The objective of this study was to evaluate the thermal resistance of *S. aureus* in various RTH sauces.

Methods: A five-strain mixture (*S. aureus* KACC11596, *S. aureus* KACC10768, *S. aureus* KACC10778, *S. aureus* KACC13236 and *S. aureus* NCCP10862) was inoculated in 5 g of pork cutlet, meat and *Carbonara* sauces at 7 log CFU/g. The inoculated samples were then exposed to 60, 65 and 70°C, and survivals of total bacteria and *S. aureus* were enumerated on tryptic soy agar and mannitol salt agar, respectively every 30 min for 2 h. Moreover, survival data of *S. aureus* were fitted to the Baranyi model to calculate death rates, and least squares means for survivals were compared using Tukey's method.

Results: Total bacteria and *S. aureus* cell counts in pork cutlet sauce significantly decreased ($P < 0.05$) without tailing effect (no reduction of bacterial cell counts) for all heating temperatures. Cell counts of total bacteria and *S. aureus* in meat sauce and *Carbonara* sauce declined ($P < 0.05$) by 2 log CFU/g after 30 min heating at 60 and 65°C, followed by very obvious tailing effect after 30 min. However, cell counts of *S. aurues* in meat and *Carbonara* sauces linearly decreased ($P < 0.05$) for 2-h heating.

Significance: The result indicates that meat sauce and *Carbonara* sauce should be appropriately heated at high temperature to destroy *S. aureus* cells.

P2-166 Development of Models to Predict Growth/No Growth Interfaces and Kinetic Behavior of *Salmonella* on Cutting Board Surfaces

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Developing Scientist Competitor

Introduction: Cutting boards are considered as a vehicle of foodborne pathogens to contaminate meat, and *Salmonella* is one of the primary foodborne pathogens in meat processing.

Purpose: This study developed models to predict growth probabilities and kinetic behavior of *Salmonella* on the cutting boards.

Methods: Polyethylene coupons (3 x 5 cm) were inoculated with *Salmonella* (5-strain mixture). These coupons were then stored at 13, 15, 20, 25, 28, 30, 33 and 35°C for 12 h, and total bacterial and *Salmonella* cell counts were enumerated ontryptic soy agar and xylose lysine deoxycholate agar, respectively, every 2 h. The 0.5 log CFU/cm^2 was used as a threshold to determine growth or no growth for 56 combinations (temperature (8) ¡¿ time (7)). These growth response data were analyzed by the logistic regression to develop the model predicting growth/no growth interfaces of Salmonella. In addition, cell counts of Salmonella were fitted to the modified Gompertz model to calculate growth rate (μ_{max}; log CFU/cm^2/h), log phage duration (LPD; h), and upper asymptote (N_{max}; log CFU/cm^2), following secondary modeling with the square root function. The model performances were validated with observed data.

Results: Total bacteria and *Salmonella* growth were observed only at more than 25°C and 28°C, respectively. However, cell counts of total bacteria and *Salmonella* on cutting board decreased ($P < 0.05$) at less than 20 and 25°C, respectively. Growth/no growth interfaces were produced at 0.1, 0.5, and 0.9 of probabilities with acceptable performance (concordance: 93.7 %, disconcordance: 5.6%). In kinetic model, μ_{max} increased as growth temperature increased, but LPD and N_{max} were similar among growth temperatures (28-35 °C). The prediction of the model was also acceptable (Bias factor: 1.03, Accuracy factor: 1.15).

Significance: The result suggests that developed models in this study may be useful in predicting growth/no growth interface and kinetic behavior of Salmonella on polyethylene cutting board.

P2-167 Mechanisms of Resistance to High Pressures by *Vibrio parahaemolyticus*

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Introduction: High pressure has been used to control *Vibrio parahaemolyticus* in seafood. Although a >300 MPa pressure can kill the pathogen, it adversely affects seafood texture. Pressures lower than this level have been shown to be ineffective in eliminating pressure-resistant *V. parahaemolyticus* from seafood.

Purpose: The objective of this project was to observe changes in cell membrane protein and fatty acid profiles as well as Na^+K^+ATPase activity of *V. parahaemolyticus* as influenced by high pressure treatments.

Methods: A pressure-sensitive strain of *V. parahaemolyticus* (ZJGSMC001) was repeatedly treated with hydrostatic pressures ranging from 80 to 250 MPa, and cells that survived the 250 MPa treatments were selected. Soluble cell member protein and fatty acid profiles of the pressure resistant derivates and their sensitive parent strains were determined using the SDS-PAGE electrophoresis and GC-MS method, respectively. The Na^+K^+ATPase activity of the strains were determined using a commercial assay.

Results: Results indicated that when exposed to a pressure of 250 MPa culturable cell counts of pressure-resistant strains was *ca*. 3 logs higher than that of pressure-sensitive strains. The pressure-resistant strains appeared to have a greater amount of soluble cell membrane protein of 35 KDa. The Na^+K^+ATPase was 83.3% more active than that of pressure-sensitive strains. The ratio of unsaturated *vs*. saturated fatty acids of cell membranes was 1.03 in the pressure-resistant strains comparing to 1.17 in the pressure-sensitive strains.

Significance: Results suggest that cell membranes of *V. parahaemolyticus* were damaged when exposed to a 250 MPa pressure. Cell membrane protein and fatty acid profiles as well as Na^+K^+ATPase activity may have played a role in mediating the resistance of *V. parahaemolyticus* to high pressures. Pressure-resistant strains may have survived the treatments through degradating soluble cell membrane proteins, enhancing the activity of Na^+K^+ATPase, and increasing the expression of saturated fatty acids.

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P2-168 Pickled Egg Production: Inactivation Rate of *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* during Acidification Step

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Introduction: Regulatory approval of commercial pickled egg production without a final heat treatment requires challenge studies based on current FDA acidified foods guidelines.

Purpose: We conducted challenge studies to verify pickled egg processing parameters.

Methods: Hard-boiled eggs were acidified in ambient temperature brine at 60/40 egg/brine ratio. Four brine treatments were studied in triplicate: 5% acetic acid (AA) and 2.5% AA, with and without 0.05% sodium benzoate. Samples were stored at 7°C until pH at the yolk center ≤ 4.6; subsequently, samples were held at ambient temperature. Egg pH was measured at 24-48 hour intervals until equilibrium pH was reached (4.0 and 4.4). Eggs and lids were challenged with separate pathogen cocktails (6 strains/serovars): *Salmonella enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*.

Results: It took 5 and 9 days respectively for the pH to fall below 4.6 for 5% and 2.5% AA-brined eggs. Sodium benzoate did not affect acidification rate ($P \geq 0.05$). No difference in pathogen die-off was observed between brines with and without preservative. *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* were undetectable by enrichment (< 1 CFU/g), with eggs in 5% AA brine at 72 hours; *S. aureus* was undetectable after 7 days. In 2.5% brine, *Salmonella* was undetectable after 10 days; no pathogens were detectable by 14 days. No pathogens were detectable on lids within 72 hours for 5% AA brines. Only *S. aureus*was detectable on lids after 72 hours in 2.5% AA brine and died off rapidly at ambient temperature.

Significance: Although pathogens studied on eggs begin die-off under refrigeration, heat treatment (ambient temperature storage) was required to reach undetectable levels; minimal inversion was adequate treatment for lids. Pickled eggs should be held under refrigeration for the length of time needed to acidify them to ≤ 4.6, and then held at ambient temperatures to ensure pathogen inactivation.

P2-169 Genes That are Affected in High Hydrostatic Pressure Treatments in a *Listeria monocytogenes* Scott A ctsR Deletion Mutant

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Introduction: *Listeria monocytogenes* is a foodborne pathogen of significant threat to public health. High hydrostatic pressure (HHP) treatment can be used to control *L. monocytogenes* in food. The CtsR (class three stress gene repressor) protein negatively regulates the expression of class III heat shock genes. In a previous study, a spontaneous ctsR *L. monocytogenes* deletion mutant 2-1 that was able to survive under HHP treatment was identified; however, there is only limited information about the mechanisms of survival and adaptation of this mutant in response to high pressure.

Purpose: To investigate which genes are affected with high hydrostatic pressure treatment in the ctsR deletion mutant 2-1.

Methods: Microarray technology was used to monitor the gene expression profiles of the ctsR mutant 2-1 under pressure treatment (450 Mpa, 3min). Gene expression changes determined by microarray analyses were confirmed by real-time RT-PCR assays.

Results: Compared to the non-pressure-treated ctsR mutant 2-1, 14 genes were induced (> 2-fold increase) in the ctsR deletion mutant whereas 219 genes were inhibited (< -2-fold decrease) by pressure treatment. The induced genes included genes encoding proteins involved in synthesis of purines, pyrimidines, nucleosides, and nucleotides, transport and binding, transcription, cell membrane, DNA and energy metabolism, protein synthesis, and unknown functions. The inhibited genes included genes encoding proteins for transport and binding, cell envelope, transcription, amino acid biosynthesis, regulatory functions, cellular processes and central intermediary metabolism.

Significance: The information concerning *L. monocytogenes* survival under HHP at the molecular level may contribute to improved HHP treatments for food processing.

P2-170 Effect of Fat Concentrations in Frankfurters on Survival of *Listeria monocytogenes* in the Gastric Fluid and Thermal Stress during Storage at 10°C

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Developing Scientist Competitor

Introduction: The relationship between fat concentrations in foods and bacterial resistance to food-related stresses has been questioned

Purpose: The objective of this study was to evaluate effects of fat concentrations of frankfurters on survival of *Listeria monocytogenes* under simulated gastric fluid and heat process, and invasion of the pathogen on Caco-2 cell.

Methods: Three frankfurters formulations (10% fat, F10; 20% fat; F20 and 30% fat, F30) were prepared, and a 10-strain mixture of *L. monocytogenes* was inoculated on frankfurters to obtain 4-5 log CFU/cm^2. The inoculated samples were then vacuum packaged, and they were stored at 10 °C for 30 days. The samples were analyzed every 10 days to evaluate survival of *L. monocytogenes* from simulated gastric fluid challenge at 37 °C for 120 min, and heat challenge at 63 °C for 60 min. Survival of total bacteria and *L. monocytogenes* were enumerated on tryptic soy agar plus 0.6% yeast extract and PALCAM agar, respectively. The isolated cells from F10, F20, and F30 were subjected to the invasion assay using Caco-2 cells.

Results: Cell counts of *L. monocytogenes* on F30 samples were higher ($P < 0.05$) than those of F10 and F20 during storage, and the pathogens on F30 samples were more resistant to heat than other treatments on day 10. *L. monocytogenes* on frankfurters with higher fat concentration showed higher ($P < 0.05$) resistance to gastric fluid. Moreover, some relationship between fat concentration and cell invasion was observed.

Significance: These results suggest that high fat concentration in frankfurters may increase resistance of *L. monocytogenes* to various stresses.

P2-171 Modeling the Combined Effect of Temperature and Relative Humidity on *Bacillus cereus* on Rice Cake (sirutteok)

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Introduction: Sirutteok, a kind of rice cake, is a very popular ready-to-eat traditional food in Korea. Sirutteok is rarely heat treated before consumption, which encourages contamination with foodborne pathogens, especially *Bacillus cereus,*according to the monitoring data and other references.

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Purpose: This study investigated the combined effect of temperature and relative humidity on growth or survival of *B. cereus* on sirutteok, and also developed predictive growth models.

Methods: Rice cake (sirutteok) samples inoculated with *B. cereus* were incubated at 5, 15, 25, 35 and 45 °C under different relative humidity (50% and 80%) for different lengths of storage periods, dependent on incubation temperature. Samples were periodically determined for enumeration according to the designed sampling intervals. The experimental data of *B. cereus* at each combined condition were collected and fitted into the modified Gompertz model to estimate the growth parameters (growth rate and lag time), which were used to develop the secondary models using polynomial equation.

Results: Temperature was much more influential than relative humidity on the growth of *B. cereus* on sirutteok. The secondary models for growth rate and lag time of *B. cereus* were developed with high coefficients of determination (0.953 and 0.902). The bias factor, accuracy factor, and the standard error of prediction, which were statistic characteristics for the external validation of the predictive models, were 0.972, 1.10, and 9.03% for growth rate, while 1.05, 1.18, and 17.32% for lag time, respectively.

Significance: The developed models described the growth data of *B. cereus* on sirutteok, and provided reliable predictions for the purpose of microbiological risk assessment of *B. cereus* on sirutteok in Korea.

P2-172 Development of Dynamic Models to Predict the Fate of *Staphylococcus aureus* in Sauces and Salad Dressing during Storage at Different Temperatures

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Developing Scientist Competitor

Introduction: Although *Staphylococcus aureus* outbreaks have occurred in various sauces and dressings, dynamic models to evaluate *S. aureus* risk in sauces have not been developed.

Purpose: This study developed dynamic models to predict the fates of *S. aureus* in sauces and dressing.

Methods: *S. aureus* 5-strain mixture was inoculated in 5 g of *Carbonara* and octopus sauce at 3 log CFU/g, and oriental dressing, bean paste sauce, *Katsuobushi* sauce, low sodium soy sauce and *Soba* sauce at 7 log CFU/g. *Carbonara* and octopus sauces were stored at 10, 15, 20, 25 and 30 °C for 15 days, and they were analyzed for *S. aureus* growth, but the other sauces were analyzed at room temperature to estimate decreases in cell counts of *S. aureus*. Growth data of *S. aureus* for*Carbonara* and octopus sauces were fitted to the Baranyi model to calculate lag phage duration (LPD; day), maximum specific growth rate (μ_{max}; log CFU/g/day) and upper asymptote (N_{max}; log CFU/g). The parameters were then fitted to secondary models, and a dynamic model was also developed for changing temperatures. The developed model was validated using observed data, and bias (*B*) and accuracy (*A*) factors were calculated.

Results: *Carbonara* and octopus sauces had *S. aureus* growth at 10-30 °C and 15-30 °C, respectively, but the other sauces had significant *S. aureus* decrease ($P < 0.05$). The LPD and μ_{max} of *S. aureus* in *Carbonara* and octopus sauces were 2.18-80.7 days and 0.02-0.45 log CFU/g/day, respectively, and *S. aureus* grew up to around 9 log CFU/g. R^2 values of secondary models were 0.732-0.975. The goodness of fit for growth model was acceptable (*A* factor: 1.03-1.07; *B* factor: 0.99-1.06), and prediction of the dynamic model showed also acceptable performance.

Significance: The developed predictive models for *Carbonara* and octopus sauces should be useful in predicting *S. aureus* growth.

P3-01 Incidence and Inactivation of *Listeria* spp. on Frozen Shrimp

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Introduction: Foodborne illness outbreaks occasionally occur as a result of microbiologically contaminated crustaceans, including shrimp. Foodborne pathogens occasionally found on shrimp include *Listeria monocytogenes*, *Salmonella* spp.,*Staphylococcus aureus*, and *Vibrios*.

Purpose: In this study the microbiological quality of frozen raw shrimp was investigated. The use of 3 intervention technologies to inactivate *Listeria* on shrimp was also investigated.

Methods: Thirty-two frozen shrimp samples, both imported and domestic, were tested for aerobic plate counts (APC at 22 and 37 °C), coliforms, and Enterbacteriaceae, using USDA Food Safety Inspection Service (FSIS) Microbiological Laboratory Guide procedures, as now FSIS is responsible for testing some aquaculture products. FSIS procedures were also used to determine the incidence of *L. monocytogenes* (LM), *Salmonella* spp., *S. aureus* on the shrimp samples. Three intervention technologies, ultraviolet light (254 nm, 0.5 to 2.0 J/cm^2), ozonated water (2 ppm, 0.25 to 2 min.), and gamma radiation (1.0 to 5.0 kGy) were used to inactivate *L. innocua*, a LM surrogate, surface inoculated onto shrimp in a pilot plant setting.

Results: The average APCs were approximately 4.06 and 3.64 log CFU/g at 22 and 37 °C, while coliforms and enteric bacteria were approximately 0.02 and 1.43 log CFU/g, respectively. None of the samples tested positive for *Salmonella*spp., *S. aureus* using the BAX-TM polymerase chain reaction detection. 7/32 tested positive for *Listeria* spp., and viable LM was recovered from 4 (12.5%) of the 32 shrimp samples. Ultraviolet light (2 J/cm^2) inactivated approximately 0.4 log CFU/g, ozonated water (2 ppm, 2 min) inactivated 1.5 log CFU/g, and 5 kGy gamma radiation inactivated > 5 log CFU/g of *L. innocua*, respectively.

Significance: Additional research is needed to determine the effect of commonly used intervention technologies on the detection and isolation of foodborne pathogens in seafood products.

P3-02 Inactivation of Foodborne Pathogen on Crawfish Tail Meat Using Cryogenic Freezing and Gamma Radiation

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Introduction: Foodborne illness outbreaks occasionally occur as a result of microbiologically contaminated crustaceans, including crawfish. Cryogenic freezing and gamma radiation are two technologies which can be used to improve the microbiological safety and shelf-life of foods. In the U.S., the majority of non-canned seafood is either frozen or previously frozen. A petition to allow irradiation of crustaceans is currently being evaluated by the U.S. Food and Drug Administration.

Purpose: There is little data on the use of commercial or pilot-scale equipment and processes to quantify the effect of freezing on foodborne pathogen survival. The purpose of this study is to evaluate the effect of cryogenic freezing and gamma radiation on the survival of foodborne pathogens in crawfish tail meat.

Methods: In this study the use of cryogenic freezing (CF) and gamma radiation (GR) were evaluated for their ability to inactivate the foodborne pathogens *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and*Vibrio* spp. inoculated on partially cooked

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crawfish tail meat. The final inoculums level on the crawfish tail meat was 6 – 7 log CFU/gm.

Results: CF (-59 °C, 3 min) resulted in 0.85, 0.71, and 0.80 log reductions of the Gram-negative pathogens *E. coli* O157:H7, *Salmonella* spp., and *Vibrio* spp., respectively, following freezing and one week storage at -20 °C. CF had no effect on the viability of *L. monocytogenes* or *S. aureus* (Gram positive). D10 values (the radiation dose needed to inactivate one log of microorganism) of the foodborne pathogens which were irradiated following one week frozen storage were 0.81, 0.58, 0.50, 0.49, and 0.36 kGy for *L. monocytogenes*, *S. aureus*, *Vibrio* spp., *Salmonella* spp., and *E. coli* O157:H7, respectively. The radiation D10 of background microflora in non-inoculated frozen tail meat was 1.58 and 1.64 kGy for mesophilic and psychrotrophic bacteria, respectively.

Significance: This research provides the seafood and radiation processing industries the necessary information to inactivate foodborne pathogens in crawfish tail meat.

P3-03 Heat Resistance of Histamine-producing Bacteria in Irradiated Tuna Loins

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Introduction: Consumption of foods high in biogenic amines leads to an illness known as histamine or scrombroid poisoning and it is commonly associated with consumption of fish. *Morganella morganii* is one of the most prolific histamine formers in certain species of fish including sardines, mackerel and tuna. *Enterobacter aerogenes* is the main histamine-producing organism in Korean fish. *M. morganii* has been shown to produce histamine when present in sufficient numbers (≥ 10^6CFU/ml) with histidine available as a substrate.

Purpose: To determine the thermal parameters of the five highest histamine-producing bacteria, *M. morganii, Raoultella planticola, Hafnia alvei, E. aerogenes,* and *Photobacterium damselae* in pre-irradiated tuna loins.

Methods: Three-strain composite for each organism was used. Tuna-samples (5 g/sample) were submerged into one bacterial composite at a time and held on ice for 30 min to allow the bacteria to adhere to the tuna loin. The final inoculum level on samples was ~ 1×10^8 CFU/g. After inoculation, samples were aseptically transferred to sterile plastic pouches, vacuum sealed, and heat treated to as many as four different temperatures for four predetermined heating times per temperature. After heating, samples were cooled down in ice slurry for ~30-40 s. To enumerate the recovered cells, each pouch was diluted using 0.1% peptone water (PW) and stomached for 2 min; further decimal dilutions were made in 9.9 ml PW before spiral plating onto tryptic soy agar (TSA). The plates were incubated for 48 h at 30 °C before enumeration. *D*-values were calculated using the GInaFiT (Geeraerd and Van Impe Inactivation model Fitting Tool) xla program.

Results: *M. morganii* was the most heat resistant histamine-producing organism tested in tuna loins ($D_{54°C}$= 8.1 ± 1.7 min, $D_{56°C}$ = 2.7 ± 1.06 min), followed by *E. aerogenes* ($D_{54°C}$ = 5.87 ± 0.78 min, $D_{56°C}$ = 0.81 ± 0.08 min), *H. alvei*($D_{54°C}$ = 2.6 ± 0.48 min, $D_{56°C}$ = 0.9 ± 0.34 min), and *R. planticola* ($D_{54°C}$ = 2.26 ± 0.24 min, $D_{56°C}$ = 0.52 ± 0.18 min).

Significance: The reported D-values for *M. morganii*, the most heat resistant histamine-producing organism tested, will help the tuna industry better understand the impact of pre-cooking temperatures on kill rates for the five histamine-forming bacteria evaluated in the study.

P3-04 Extraction of Enteric Virus Indicator from Seawater Using Activated Carbon

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Developing Scientist Competitor

Introduction: Enteric virus-contaminated shellfish represents a significant health threat to shellfish consumers as well as an economic threat to the seafood industry. Male specific bacteriophage MS2 has been identified as a suitable indicator for water-borne enteric virus outbreaks, and its presence indicates a recent contamination. There is an urgent need for the development of rapid detection methods that enable efficient detection of viruses in seawater samples.

Purpose: To investigate if activated carbon can be used for extraction of MS2 bacteriophage from seawater.

Methods: The effects of pH (4, 5, 6, 7, 8, and 9), salinity (0, 10, 20, 30, and 40 ppt) and contact temperature (4 °C, 20 °C and 37 °C) on the absorbance efficiency of activated carbon were investigated in artificial seawater. MS2 bacteriophage at 1E8-1E9 PFU were inoculated into 500 ml of artificial seawater and 1 g of activated carbon was added. After 3 h of constant stirring, activated carbon was separated from the seawater, incubated with 1 ml of trypsin-EDTA solution for 2 h at room temperature to release MS2 bacteriophage from the activated carbon, and RNA was extracted. qRT- PCR was conducted to determine the PFU of MS2 bacteriophage released from the activated carbon.

Results: Results indicated that temperature has a significant effect on the absorbance efficiency of activated carbon. Warmer temperature provides better efficiency for activated carbon. In zero salinity water, the pH has no significant effect on the absorbance efficiency of activated carbon, regardless of the temperature. Activated carbon has significant higher absorbance efficiency in seawater than zero salinity water. Seawater with salinity of 10 ppt at a contact temperature of 37 °C was proven to provide the best absorbance efficiency for activated carbon.

Significance: The use of activated carbon has the potential to be developed into a rapid concentration and detection method for enteric water-borne viruses.

P3-05 Effects of Antimicrobial Peptides on *In Vitro* and *In Vivo* Growth and Survival of *Vibrio* spp.

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Introduction: The application of antimicrobial peptides (AMPs), and nisin in particular, is increasingly being employed by the food industry to inhibit and/or eliminate Gram-positive pathogens. However, the effectiveness of AMPs against Gram-negative organisms has traditionally been limited. Fortunately, recent research has shown that certain chemical or physical stressors, leading to increased permeability of the Gram-negative outer membrane, increase the effectiveness of AMPs against these bacteria.

Purpose: These studies examined the activity of the AMPs hepcidin and nisin against *V. vulnificus*, *V. parahaemolyticus* and *V. cholerae* with and without the application of chemical or physical stressors *in vitro* and in live oysters.

Methods: *In vitro* bacterial growth inhibition by hepcidin and nisin with and without EDTA or cold shock was evaluated in microtiter plates and in culture flasks, respectively. Population changes were measured using plate counts and/or optical density. The effectiveness of nisin with and without cold shock and/or EDTA was also tested against *V. vulnificus* in live oysters. These studies evaluated reductions in both the natural bacterial populations as well as populations in a previously established oyster infection model.

Results: *In vitro* growth of *V. vulnificus* was inhibited by hepcidin at various inocula (10^3-10^5 CFU/ml), but only at higher hepcidin concentrations (50, 75 and 100 µM). Exposure to nisin alone or in conjunction with EDTA had no effect on*in vitro* growth or survival of any *Vibrio spp* evaluated. However, concomitant cold shock, EDTA and nisin exposure resulted in significant (*P* ≤ 0.035) reductions in culture concentrations for all

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three *Vibrio* spp. Natural populations of *V. vulnificus* in oysters also showed in a reduction (but not significant) of about 1.0 log CFU/ml in response to the synergistic effect of cold shock, EDTA and nisin.

Significance: Results from these studies support the potential application of a hurdle-type postharvest processing treatment for oysters.

P3-06 *Withdrawn*

P3-07 Prevalence, Characterization, and Antimicrobial Susceptibility of *Salmonella* Gallinarum in the Contents of Shell Eggs

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Introduction: Salmonellosis is one of the most widespread infectious diseases in the world. Poultry-derived products, especially eggs, are commonly consumed foods that may occasionally be contaminated with *Salmonella*.

Purpose: This study was conducted to evaluate the extent of *Salmonella* contamination in shell eggs in South Korea.

Methods: A total of 7,700 shell eggs (385 pooled samples of 20 eggs) were collected in the period 2010-2011, and *Salmonella* isolation was performed by the culture method according to the FDA's *Bacteriological Analytical Manual* (BAM). The surfaces of eggs were disinfected with solution consisting of three parts 70% alcohol to one part iodine/potassium iodide for at least 10 seconds. And egg contents were obtained by cracking eggs aseptically. A total of 385 different bulk pools made of 20 whole shell egg contents each were prepared. Egg contents placed in sterile bags were mixed manually and incubated for 96 h at room temperature (20-24 °C). The sample (25 ml) mixed with 225 ml of sterile TSB were followed by incubation at 37 °C for 24 h. The enriched TSB culture (0.1 ml and 1 ml) were transferred into 10 ml of RV and MKTTn and incubated at 37 °C and 42 °C for 24 h, respectively. A loopful of RV and MKTTn enrichment culture was streaked onto XLD Agar plates. The presumptive colonies with a positive result were confirmed as *Salmonella* with VITEK2(bioMérieux) and *Salmonella*-specific real-time PCR.

Results: *Salmonella* was detected in 27 of 385 pooled samples of shell eggs. All *Salmonella* isolates were typed as *Salmonella* Gallinarum. The isolated *Salmonella* strains were also investigated for antibiotic resistance and showed similar antibiotic resistance patterns. Egg isolates were further characterized with the automated repetitive sequence-based PCR (rep-PCR) system (DiversiLab). The rep-PCR produced 2 different genetic groups among these isolates, and isolates within the same group did show clear relationships expecially with respect to the efficacy.

Significance: The strains of *S.* Gallinarum isolated from eggs seems to be different from the vaccine strain being used in Korea. It may indicate that outbreaks of SG in layer chickens still occur in South Korea despite extensive vaccination since 2001. It seems that the present vaccines still need to be improved, expecially with respect to efficacy.

P3-08 Comparison of Two Commercial Real-Time PCR Systems with Culture Methods for the Detection of *Salmonella* spp. in Environmental and Fecal Samples of Poultry

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Introduction: *Salmonella* are an important cause for severe human gastroenteritis after consumption of contaminated poultry products, especially eggs and meat. European regulations are aiming to reduce *Salmonella* prevalence in primary production of poultry to less than 1%.

Purpose: The purpose of this study was to evaluate the foodproof® *Salmonella* detection kit for the detection of *Salmonella* in sample types being mandatory for monitoring primary production such as boot socks, feces, feed and dust.

Methods: The study compared the efficiency of the foodproof® *Salmonella* detection kit and Bax® PCR Assay *Salmonella* system with standardized culture methods (EN ISO 6579:2002 – Annex D) for the detection of *Salmonella* spp. in poultry samples. For evaluation four sample matrices (feed, dust, boot swabs, feces) directly from poultry flocks as well as artificially spiked samples of the same matrices were used. All samples were tested first for *Salmonella* spp. using culture methods as the gold standard. Furthermore, all methods were evaluated in an annual ring-trial of the National *Salmonella* Reference Laboratory of Germany.

Results: *Salmonella* detection in the matrices feed, dust and boot swabs led to comparable results of both PCR systems whereas the results from feces differed markedly. Furthermore, the quality, especially the freshness, of the fecal samples had an influence on the sensitivity of the real-time PCR and culture methods results. In fresh fecal samples an initial spiking level of 100 CFU/25 g *Salmonella* Enteritidis was detected. Dry fecal samples allowed the detection of 14 CFU/25 g. Both real-time PCR protocols appear to be suitable for the detection of *Salmonella* spp. in all four matrices.

Significance: RT-PCR reduces time to result to two days only compared to classical culture enabling, e.g., faster restocking or product release.

P3-09 A Novel Multiplex Real-Time PCR Method for Rapid Detection and Serotyping of *Salmonella* Enteritidis and Typhimurium

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Introduction: In Europe, food regulation was recently reinforced to prevent poultry meats adulterated with either *S.* Enteritidis or *S.* Typhimurium from being marketed. While the current Kauffmann-White serotyping method requires 4/5 days to assess the presence of these pathogens, a new method was developed as an alternative, single-tube real-time PCR method allowing their simultaneous and specific detection within two days.

Purpose: This study aimed at evaluating the performances of the iQ-Check *Salmonella* E&T kit on food and environmental samples, the detection limits (LD), and the specificity.

Methods: Twenty-three samples were artificially spiked with low amounts of either *Salmonella* Enteritidis or Typhimurium and subjected in parallel to both iQ-Check *Salmonella* E&T and iQ-Check *Salmonella* II methods. For LD, genomic DNA was extracted from pure strains and its concentration evaluated by $OD_{260\ nm}$. Serial dilutions were subsequently assayed by PCR. For inclusivity study, 45 *Salmonella* Enteritidis and Typhimurium strains were tested.

Results: All 23 spiked samples yielded positive results. Detection limit was estimated between 2 and 10 genome units per PCR well for both serotypes. Inclusivity was validated on all 19 *Salmonella* Enteritidis and 26 *Salmonella* Typhimurium strains.

Significance: The test allows a faster release of the products and the reduction of the number of samples to be confirmed by the standard method. Additional evaluations are currently ongoing in different laboratories. The method will also allow the detection of the *Salmonella* Enteritidis serovar only, to better address the needs of the US eggs industry.

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P3-10 Comparison of Detection Methods for *Salmonella* in Egg: Individual Sampling vs. Pooling Sampling

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Introduction: Salmonellosis is an important foodborne disease worldwide. The causative agent, *Salmonella*, is transmitted by many food vehicles, especially eggs. Because *Salmonella* cells are typically present in low concentrations and only sporadically in individual eggs, sensitive isolation methods and analysis of large numbers of eggs are necessary.

Purpose: To compare sampling and pre-enrichment methods for detection of *Salmonella* in eggs.

Methods: A total of 400 different bulk pools were each prepared from the contents of 20 whole shell eggs. Eggs were cracked aseptically and the egg contents were agitated in a stomacher bag and thoroughly mixed manually. Each bulk pool was artificially inoculated with 1 ml of *Salmonella* in PBS at 88, 68, 53, and 20 CFU per egg pool. Pre-incubation conditions were: (1) no pre-incubation (2) pre-incubation at room temperature for 4 days (3) pre-incubation at 37 °C for 1 day (4) pre-incubation at 41 °C for 1day. All samples were 25 ml. The isolation method of *Salmonella* from egg contents was according to the FDA's Bacteriological Analytical Manual (BAM). Presumptive *Salmonella* isolates were identified by a *Salmonella*Enteritidis-specific PCR using the *sef14* gene target.

Results: Detection rate of Group (1) was 7/20 (35%) in 88 CFU/pool, 3/20 (15%) in 68 CFU/pool, 0/20 (0%) and 0/20 (0%) in 20 CFU/pool; Group (2) was 18/20 (90%) in 88 CFU/pool, 18/20 (90%) in 68 CFU/pool, 20/20 (100%) in 53 CFU/pool and 5/20 20 CFU/pool; Group (3) was 20/20 (100%) in 88 CFU/pool, 20/20 (100%) in 68 CFU/pool, 9/20 (45%) in 53 CFU/pool, 7/20 (35%) in 20 CFU/pool; and Group (4) was 18/20 (90%) in 88 CFU/pool, 20/20 (100%) in 68 CFU/pool, 18/20 (90%) in 53 CFU/pool, 9/20 (45%) in 20 CFU/pool.

Significance: Use of a 20-egg pool in pre-enrichment was very sensitive compared to the 25 ml direct testing method. In the FDA BAM methods, groups B, C, and D had similar results in different pre-incubation conditions. A method involving a 1-day pre-incubation at 41 °C will save time for detection.

P3-11 Statistical Distribution of Human Error in Positioning Temperature Probes in Meat Patties for Thermal Process Validation

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Introduction: Thermal processing is the primary intervention step for ready-to-eat meat and poultry products. Validation of such processes depends on placement of a temperature probe in the "cold spot" of the product, with the resulting data used either to confirm the target endpoint temperature or compute pathogen reduction (e.g., *Salmonella*). However, the inherent uncertainty in such procedures has not been previously documented, but would affect process validation calculations and ultimately product safety.

Purpose: The objective of this project was to quantify the contribution of human error to temperature probe placement in meat patties, which subsequently affects error in endpoint temperature and lethality determination.

Methods: Two different groups of human subjects were tested. The first group (n = 12) consisted of laboratory personnel with experience working with meat patties and temperature probes. The second group (n = 20) consisted of individuals solicited from the general university population. Each subject was presented with three beef patties (thickness ~ 1.2 cm; diam ~ 12 cm) and three rigid temperature probes connected to wire leads. The subjects were instructed to insert one probe through the side of each patty so that the tip was in the geometric center. Each tray was then frozen to fix the probe positions. The samples (n = 96) were subsequently scanned via computed tomography (CT). The CT images (resolution 0.625 x 1.00 mm) were processed using Matlab to identify the centroid of each patty and the location of the probe tip relative to the centroid.

Results: For the lab and public groups, respectively, the mean (± standard deviation) vertical errors in position (i.e., bias), were -1.5 ± 2.9 and -2.8 ± 3.3 mm; the mean absolute vertical errors were 2.7 ± 1.8 and 3.1 ± 2.6 mm; and the mean total distances from the centroid were 6.9 ± 4.0 and 10.5 ± 6.7 mm.

Significance: We have previously reported that temperature probe errors of 3 mm in a convection cooked patty can yield lethality errors of ~5 log, or correspond to increased required endpoint temperature or cooking time of 9 °C or 40 s. Overall, human error in temperature probe position can contribute significantly to the inherent uncertainty in thermal process validations, and should be accounted for in such analyses.

P3-12 Antibiotic Resistance and Virulence Potentials of Shiga Toxin-producing *Escherichia coli* Isolates from Retail Meat Products in Korea

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Introduction: Shiga toxins (Stxs) are the AB5-type bacterial toxins with a potent cytopathic effect on mammalian cells. Therefore, contamination of highly-pathogenic Stx-producing Esherichia coli (STEC) among retail meat products is an important threat to public health and food safety.

Purpose: Prevalence and pathogenic potentials of STEC isolates from retail meat products in Korea were evaluated.

Methods: During the years 2008-2009, a total of 912 retail meat products including beef, pork and chicken were collected in Korea. *E. coli* was isolated according to the Korean Food Standards Codex. Further identification of STEC was done by detection of the *stx* genes and O-serotyping. Antibiogram of individual STEC isolates was determined by disk diffusion or minimal inhibitory concentrations according to the guideline of clinical laboratory standard institute. The polymerase chain reactions were employed to detect the previously defined virulence genes in STEC, including Stx1 and 2 (*stx1 & 2*), enterohemolysin (*ehxA*), intimin (*eaeA*), STEC autoagglutination adhesin (*saa*) and subtilase cytotoxin (*subAB*). Stx production was analyzed quantitatively by a reverse phase latex agglutination (RPLA) kit.

Results: Among the 469 *E. coli* strains isolated during the experimental periods, 12 (2.6%) isolates contained the *stx* gene and belonged to 12 O-serogroups. They all carried *stx1* and *eae* genes, but none of them had *stx2*, *saa*, and *subAB*genes. Six (50.0%) STEC isolates possessed the *ehxA* gene, which are known to be plasmid-encoded. As expected, the antibiogram profiles showed that some STEC strains, especially pork and chicken isolates, displayed a multiple drug resistance phenotype. The RPLA analysis revealed that all the STEC isolates produced Stx1 only at the undetectable levels.

Significance: Prevalence of STEC isolates and their pathogenic properties have been analyzed in Korea for the first time, implying that a unique pattern(s) might be present among the Korean STEC isolates.

P3-13 Development and Model Testing of Anti-mortem Screening Methodology to Predict Prescribed Drug Withholds in Heifers

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Introduction: A simple, cow-side test for the presence of drug residues in live animal fluids would provide useful information for tissue drug residue avoidance programs. Live animal tests have the potential to allow verification that an individual animal is free of drug residues before sale for human consumption.

Purpose: Adapt the Kidney Inhibition Swab (KIS) test to detect antibiotics in live animal fluids, urine and serum. Determine the fluids' ability to predict the withhold time of sulfadimethoxine (SDM).

Methods: Feed-lot heifers were treated with SDM intravenously at 55 mg/kg. Initially 3 animals were dosed. Urine was collected daily for 5 days. Blood, spun down to serum, and saliva were collected pre, day 1 and 4 post-treatment. In the 2nd dosing 12 treated animals had urine and serum collected daily for 6 days. Samples were tested by liquid chromatography and with KIS-modification (Charm Sciences, Inc.).

Results: In the 1st dosing, both urine and serum samples at 24 hours were KIS positive. With day 2 and 3 urine samples 1 of 3 cows were positive. The 4th day urine samples were negative, while 2 of 3 serum samples were positive. HPLC indicated the presence of parent compound in serum and the presence of parent and metabolite in urine. Saliva sampling and testing was too variable. In the 2nd dosing experiment the urine samples were 80% KIS positive on days 1 and <5% positive on days 2, 3 and 4. Serum samples were 100% positive on days 1 and 2; 75% positive on day 3; 67% positive on day 4; 50% positive on day 5 and 30% positive on day 6.

Significance: The recommended withhold time of SDM is 5 days post treatment. Serum tests were more predictive than urine in detecting treated animals. Other drug studies are needed to determine if this relationship is unique to the drug used. Understanding incurred-drug-residue relationships between tissue, urine and serum samples are important to acting upon screening results.

P3-14 Variation in the Microbiological Quality of Cooked Meat After Slicing in Retail Premises

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Introduction: In Wales, United Kingdom, in 2005, there was a significant outbreak of foodborne disease caused by *E. coli* O157 that was microbiologically and epidemiologically linked to contaminated cooked meat. In response to this outbreak, there have been a number of studies in Wales focusing on this type of product.

Purpose: The goal of this particular project was to ascertain the extent to which, in retail premises, the unpacking, handling and slicing of meat joints, and the subsequent repacking of the sliced meat affected the microbiological quality of the final, sliced product. Specifically, the aim of the project was to observe if the handling and slicing of meat joints within this type of premise increased the hazard to the public.

Methods: A total of 55 premises (butchers and butchery departments in supermarkets) that produced packets of sliced, cooked meat from meat joints for retail sale were visited. Samples were taken pre and post slicing by environmental health officers and delivered to the nearest public health laboratory where they were examined for aerobic colony count, Enterobacteriaceae, *Escherichia coli*, *Listeria* and *Salmonella* using international standard methods (ISO methods).

Results: Microbiological results were compared to current UK guidelines for the quality of ready-to-eat food. Comparison of results between pre and post slicing found a decrease in unsatisfactory rate from 6.3% to 4.7% for ACC, an increase for both Enterobacteriaceae (1.6 to 3.9%) and *E. coli* (1.6 to 2.4%) and no *Salmonella* or *Listeria* in any samples.

Significance: It can be concluded that for the premises visited during the survey, that the unpacking of the joint, handling, slicing and packaging of the slices did not significantly increase the microbial hazard within this type of product.

P3-15 Microbiological Performance of a High Pressure System in Comparison with Trimming to Control *Salmonella*, *Campylobacter* and Indicator Microorganisms in Poultry Carcasses

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Introduction: *Salmonella* and *Campylobacter* are recognized as pathogens of public health importance as well as because of the economic impact they have in international trade. There is a zero tolerance for apparent gastrointestinal contamination (AGC) in poultry carcasses. Although a critical control point, Brazilian slaughterhouses are not allowed to use other procedure or technology than the trimming of the carcass with AGC.

Purpose: To evaluate the efficiency of a High Pressure System (HPS) with water to remove the AGC from poultry carcasses and to compare its influence on the microbiological quality.

Methods: A pilot study was developed in a slaughter with a level of 5% of carcasses with AGC after evisceration, but before chilling (review step). Six groups of carcasses, representing three treatments were collected (500 carcasses each). HPS was installed prior to the review step and operated with 1.5 l of water/carcass (10 kgf/cm^2). Detection and enumeration of *Salmonella* and *Campylobacter* were conducted using ISO methods and indicators aerobic mesophilic, *E. coli* and*Enterobacteriaceae* using 3M Petrifilm. Validation of HPS was conducted in four slaughterhouses with AGC up to 12% (3,250 carcasses). For validation the Bax System (DuPont Qualicon) was used for *Campylobacter* analysis.

Results: Distribution of *Salmonella* and *Campylobacter* in poultry carcasses was heterogeneous amongst the different treatments and the presence of these pathogens was not related with the presence of AGC on the carcasses. No statistical difference ($P < 0.05$) was observed between the samples indicating that AGC presence on the carcasses do not affect negatively the microbiological quality of the carcasses. HPS showed several advantages over trimming.

Significance: The results of this study were used by the Brazilian government as the scientific basis for a new regulation, published at the end of 2011, allowing the use of water to remove AGC from chicken carcasses.

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P3-16 Presence of Shiga-toxin Producing *Escherichia coli* in Small and Very Small Beef Processing Plants and Resulting Beef Products Detected by a Multiplex Polymerase Chain Reaction Assay

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are pathogens attributed to numerous foodborne illnesses resulting in gastrointestinal disease of varying severity, including hemolytic uremic syndrome (HUS) in humans. Cattle and consequently, beef products are considered a major source of STEC. *E. coli* O157:H7 has been regulated as an adulterant in ground beef since 1996.

Purpose: The USDA-Food Safety and Inspection Service has proposed that 6 additional STEC (O145, O121, O111, O103, O45 and O26) will be regulated as adulterants in beef trim and raw ground beef, beginning in March 2012. It is the goal of this research to determine if small and very small beef processing plants are a potential source of STEC.

Methods: In this study, carcass swabs, hide swabs, ground beef and environmental samples from small and very small beef processing plants were obtained from October 2010 to December 2011 to determine the presence of STEC. A multiplex polymerase chain reaction assay was used to determine the presence of STEC O157, O145, O121, O113, O111, O103, O45 and O26 in the samples.

Results: Results demonstrated that 35.0% (71/203) of the carcass samples, 56.6% (154/272) of the environmental samples, 85.2% (23/27) of the hide samples and 17.0% (20/118) of the ground beef samples tested positive for one or more of the serogroups. However, only 0.044% (9/203) of the carcass samples, 0.074% (20/272) of the environmental samples, 0% (0/27) of the hide samples and 0% (0/118) ground beef samples tested positive for both the serogroup and Shiga toxin genes.

Significance: Based on this survey, small and very small beef processors may be a source of the 6 non-O157:H7 STEC. The information from this study may be of interest to regulatory officials, researchers, public health personnel, and the beef industry that are interested in the presence of these pathogens in the beef supply.

P3-17 Use of High Hydrostatic Pressure to Extend the Shelf-life of Vacuum-packaged Caiman Alligator (*Caiman yacare*) Meat during Chilling Storage

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Introduction: Vacuum packaging (VP) is a method widely used to increase the shelf life of chilled meat. High hydrostatic pressure (HHP) is proposed as a post-processing intervention technique, serving as an additional hurdle to enhance the microbiological quality and safety of foods. The combination of VP and HHP may extend the shelf life of the caiman chilled meat while keeping its freshness and safety for a longer period.

Purpose: This study aimed at evaluating the use of HHP to extend the shelf life of vacuum-packaged caiman meat during chilling storage conditions.

Methods: Tails of caiman alligator originated from an inspected abattoir were acquired. The tails were vacuum-packaged and kept under refrigeration (4 ± 1°C) until used. Caiman meat was cut into steaks of approx. 25 g, vacuum packaged in plastic bags and subjected to four different treatments: control (no HHP), 200Mpa, 300Mpa and 400Mpa for 10 min. The packages of caiman meat were stored at 4 ± 1°C for up to 90 days. During different time intervals, 25 g of the samples were collected and analyzed for total aerobic microorganisms (mesophilic and psychrotrophic), lactic acid bacteria and detection of *Salmonella*. The methods were based on preconized by APHA and the counts of microbial groups were compared using Tukey's test.

Results: The combination of VP and HHP (400Mpa) resulted in a significant reduction of counts of total aerobic microorganisms (mesophilic and psychrotrophic) and lactic acid bacteria (> 3 log CFU/g) even after 90 and 60 days of chilled storage, respectively ($P < 0.05$). *Salmonella* was detected in control samples of vacuum-packaged caiman meat, while no recovery of this pathogen was obtained from HHP-treated samples.

Significance: The extension of the shelf life of vacuum-packaged caiman meat and enhancement of its microbiological safety may facilitate the commercialization and consumption of the product. A further step of the study includes the validation of HHP processing conditions for inactivation of *Salmonella*.

P3-18 Bactericidal Characteristics of Lactic Acid and Levulinic Acid Plus Sodium Dodecyl Sulfate in Pure Culture and Comparison of Different Intervention Approaches for Inactivation of Shiga Toxin-producing *Escherichia coli* on Beef Trim

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a group of foodborne human pathogens that can cause hemorrhagic colitis and hemolytic uremic syndrome in humans.

Purpose: The purpose of this study was to: (1) determine in pure culture the bactericidal activity of 3% lactic acid and 0.5% levulinic acid plus 0.05% sodium dodecyl sulfate (SDS) on STEC and (2) compare the efficacy of different treatment application methods, including spraying, immersion with pressure by hands and immersion without pressure, to inactivate *E. coli* O157:H7 on beef trim.

Methods: Lactic acid and levulinic acid and sodium dodecyl sulfate (SDS) were tested individually or in combination at different concentrations at 21°C for their killing effect on different isolates of STEC. Beef (25-35% fat content, cut into 10 x 10 x 7.5-cm portions) was inoculated by immersion with 10^{7-8} CFU *E. coli* O157:H7/ml for 1 min and mixed by hand with uninoculated beef trim at a ratio of 1:5 for 2 min. The inoculated beef was held in a laminar hood for 20 min before treatment. Treatments were applied by the methods described above.

Results: Applied to pure cultures, 3% lactic acid treatment for 5 min at 21°C reduced *E. coli* O26:H11, O45:H2, O111:H8, O103:H2, O121, O145:NM, and O157:H7 populations by 2.5, 1.9, 1.0, 2.8, >6.5, 4.3, and >6.7 log CFU/ml, respectively. However, treatment with 0.5% levulinic acid plus 0.05% SDS for <1 min at 21°C reduced all STEC by >6 log/ml reduction to undetectable populations. The spray treatment was least effective in reducing *E. coli* O157:H7 counts, providing a 0.5 and 1.0 log/CFU/sample reduction, whereas immersion without pressure provided a 1.0 and 1.5 log/CFU/sample reduction, and immersion with pressure provided an additional 0.5 and 1.0 log/CFU/sample reduction.

Significance: Results revealed that an immersion treatment with pressure was the most effective in reducing *E. coli* O157:H7 counts on contaminated beef trim.

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P3-19 Concentrations and PFGE Profiles of *Salmonella* Serovars on Poultry from Retail Markets in Seattle, Washington: 2011–2012

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Developing Scientist Competitor

Introduction: Poultry has been identified as one of the major sources of *Salmonella,* with estimates ranging from 10% to 22% of total salmonellosis cases. Despite several advances in the industry and new performance standards, salmonellosis in the population has not declined over the last ten years.

Purpose: The objective of this study was to conduct a year-long market survey of *Salmonella* on raw poultry to help bridge the data gaps needs for use in a Quantitative Microbial Risk Assessment (QMRA). The main objectives include: determine the prevalence and concentrations of *Salmonella* species between various chicken parts and forms, and genetically characterize recovered *Salmonella* isolates.

Methods: A total of 1100 raw, fresh poultry carcasses from retail markets in Seattle, Washington, were included in the study. After an overnight incubation of 350 grams of samples at 42 ± 0.2°C in modified tryptic soy broth, a polymerase chain reaction (PCR) method was used to screen for *Salmonella*. The immunomagnetic separation procedure followed by a conventional culture-based method was used for the recovery of *Salmonella*. The most probable number method (MPN) and pulsed field gel electrophoresis (PFGE) were used for enumeration and to assess genetic relatedness of the isolates, respectively.

Results: Eight percent of the samples were positive for *Salmonella*. No seasonal variations in percent-positive rates and concentrations of *Salmonella* were observed. Percent-positive rates varied by processing establishments, processing types and sample forms. A significant portion of the positive samples had < 30 MPN/100 grams. There is an overlap in the genetic variability of *Salmonella* recovered from different establishments and by seasons.

Significance: A single prevalence rate of *Salmonella* on carcasses may not adequately describe the variability in risk. Therefore future QMRAs will need to consider the variability in the contamination rates of various chicken parts, forms and processing types.

P3-20 Resistance of *Escherichia coli* O157:H7 and Other Shiga Toxin-producing Non-O157 *Escherichia coli* To Ultraviolet Treatment

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is one of the important foodborne pathogens that is known to cause severe life threatening health conditions like HUS (Hemolytic Uremic Syndrome). Though it is linked to a wide variety of foods, beef is a predominantly identified source of serious *E. coli* O157:H7 and non-O157 outbreaks.

Purpose: The purpose of the current study was to find non-thermal solutions to enhance beef safety using ultraviolet radiation. Current research aims at studying the resistance patterns of various *E. coli* O157:H7 strains to UV treatment. This study also compares the resistance of the cocktails of serotypes *E. coli* O26, O45, O103, O104, O111, O121 and O145 to UV.

Methods: Five individual strains of *E. coli* O157:H7 suspended in phosphate buffered saline (PBS, pH 7) were subjected to the UV radiation (1.04 mW/cm^2 intensity) for different time periods ranging from 20 seconds to 180 seconds. *E. coli*O26, O45, O103, O104, O111, O121 and O145 cocktails of strains were also prepared and subjected to UV radiation as above. The treated solutions were appropriately diluted and plated on the tryptic soy agar (TSA) and enumerated.

Results: The results show that among the 5 *E. coli* O157:H7 strains tested, strain 5 (human isolate) was the most resistant and followed by E009 ≥ 932 ≥ 1 ≥ 4. *E. coli* O104 was the most resistant serotype among the 7 *E. coli* serotypes tested then followed by O45 ≥ O103 ≥ O121 ≥ O26 ≥ O111 ≥ O145.

Significance: Results revealed that while 180-second treatment was enough for reducing all five strains of *E. coli* O17:H7 to non-detectable levels except for *E. coli* O157:H7-5 which is a human isolate which required 210 seconds.

P3-21 Reduction of "Big 6" Non-O157 STEC on Chilled Beef Sub-primals Using 5% Lactic Acid

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Developing Scientist Competitor

Introduction: The recent announcement by the USDA-FSIS of six non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in ground beef as adulterants warrants re-assessment and validation of current interventions.

Purpose: To evaluate the reduction efficacy of 5% lactic acid on the "Big 6" non-O157 STECs on chilled beef sub-primals.

Methods: Two briskets were randomly assigned to each of 3 treatments consisting of a positive control, sterile water, or 5% lactic acid and submerged into an inoculum consisting of the "Big 6" non-O157 STEC serotypes (O26, O11, O103, O121, O45, O145) for 1 minute before removal and placement onto a rack to facilitate pathogen attachment for 30 minutes. Positive controls were sampled and remaining briskets were sprayed with either sterile, room temperature water or 5% lactic acid. A 50 cm^2 area was swabbed, stomached at 230 rpm for 1 minute and serially diluted and plated onto MacConkey agar-tryptic soy agar overlay to facilitate injured cell recovery. Manual plate counts were conducted to assess efficacy and data analyzed using SigmaStat®.

Results: Microbial counts were converted to CFU/cm^2 and the positive control was determined to be 2.5 log CFU/cm^2. Treatment averages for water and lactic acid were 1.91 and 1.96 log CFU/cm^2, respectively. Statistical analysis was conducted using the two-way analysis of variance function of SigmaStat® 3.5. Pairwise multiple comparisons indicated statistical differences between Positive Control and Water ($P < 0.008$) and Positive Control and Lactic Acid ($P < 0.009$), but no differences were detected between Water and Lactic Acid ($P > 0.05$). No differences were detected between repetitions ($P > 0.05$).

Significance: These data detected no differences in reduction efficacy between water and lactic acid treatments but both were significantly different from the control. Water and 5% lactic acid may be an efficacious intervention against non-O157 STECs on beef subprimals. A longer contact time may increase the efficacy of the 5% lactic acid.

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P3-22 Classification of Ready-to-Eat (RTE) Meat and Poultry Products within the Public Health Information System (PHIS)

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Introduction: PHIS is a novel, web-based information technology system designed to improve the collection and reporting of inspection verification data, noncompliance reports, and other activities within FSIS-regulated establishments. In the course of developing PHIS, it was necessary to devise a new classification system for RTE meat and poultry products. With this new classification, FSIS can better categorize RTE products and identify risks from particular types of products.

Purpose: To describe the new RTE product classification system developed for PHIS.

Methods: Before PHIS, RTE product classification basically came from two sources: A process-based classification system used by the testing labs, and information from FSIS Form 10,240-1. The new PHIS classification consisted of a combination of HACCP categories and an expert elicitation, supplemented by analysis of historical RTE data.

Results: The initial RTE product classification established 4 basic categories: Acidified/fermented, dried, salt-cured and fully cooked. Analysis of the product types within the 'fully cooked' category resulted in a further classification of that category into 7 product groups: 1) Hotdog, 2) sausage, 3) salad/spread/pate, 4) meat/non-meat multicomponent, 5) patty/nugget, 6) diced/shredded and 7) other. These product groups incorporated elements of both the prior process-based and 10,240-1-based systems. It was recognized that not all products conveniently fit a particular category or group. Accordingly, a flowchart was developed to select the single best product category/product group in which a given product might be classified based on the overall microbial lethality, preservation and production methods, while being aware that mixed, multiple production methods can occur. Subsequent to integration into PHIS, descriptions of the new classification system are being included in updated Directives and *Listeria* Compliance Guidelines.

Significance: Proper RTE product classification is essential for determining pathogen (*Listeria* and *Salmonella*) percent positive rates as a function of sample product type, thus yielding insights into the potential contamination risks associated with these products.

P3-23 Association of *Campylobacter* spp. Levels between Chicken Grow-out Environmental Samples and Processed Carcasses

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Introduction: *Campylobacter* spp. have been isolated from live poultry, the poultry production environment, poultry processing facilities, and raw poultry products. Further research is needed to understand pathogen transfer from production to processing. The detection of *Campylobacter* using both quantitative and qualitative techniques would provide a more accurate assessment of pre- or post-harvest contamination.

Purpose: Environmental sampling in a poultry grow-out house, combined with carcass rinse sampling from the same flock, may provide a relative assessment of *Campylobacter* contamination and transmission.

Methods: Air samples (collected onto gelatin filters), fecal/litter samples (from disposable boot covers), and sponge samples (feed pans and drink lines) were collected from four commercial chicken grow-out houses. Birds from the sampled house were the first flock slaughtered the following day, and were sampled by post-chill carcass rinses. Quantitative and qualitative detection methods were used to determine *Campylobacter* contamination in each environmental sample and carcass rinse.

Results: *Campylobacter*, from Campy-Cefex agar plates after enrichment, was detected from 27% (32/120) of house environmental samples and 37.5% (45/120) of carcass rinse samples. All sample types from each house included at least one positive sample except for the air samples from House 2. Samples from House 1 and associated carcass rinses accounted for the highest total of *Campylobacter* positives (29/60). And, the fewest number of *Campylobacter* positives, from both house environmental (4/30) and carcass rinse samples (8/30) were detected from Flock 2.

Significance: Environmental sampling techniques provide a non-invasive and efficient way to test for foodborne pathogens. Correlating qualitative or quantitative *Campylobacter* levels from house environmental samples and post-chill carcass rinse samples may enable the scheduled processing of flocks with lower pathogen incidence or concentrations, as a way to reduce post-slaughter pathogen transmission.

P3-24 Thermal Inactivation of *Listeria monocytogenes*, *Salmonella* and Shiga Toxin-producing *Escherichia coli* in Ready-to-Eat Roast Beef

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Introduction: USDA, FSIS Appendix A is widely used as validation support for thermal processes of processed meats, but its time-temperature tables were developed only for *Salmonella* in roast, cooked, and corned beef. Pathogen- and product-specific time-temperature tables are necessary to improve validation of thermal processes.

Purpose: To determine the temperature-death times of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *E. coli* (STEC) in ready-to-eat roast beef at 54.4, 60, 65.6, and 71.1 °C.

Methods: Ground roast beef (containing 1.0% salt, 0.35% sodium phosphates, 0.75% sugar, 20% water) was inoculated with 8 log CFU/g *L. monocytogenes* or *Salmonella* (5-strain mix) or STEC (7-strain mix). One-g portions (0.5-1.0 mm in moisture-impermeable vacuum pouches) were heated at one of four temperatures (54.4, 60, 65.6, or 71.1 °C) in a water bath. Triplicate samples were immediately removed and chilled to 4 °C when meat reached target temperature and at seven additional times. Surviving *L. monocytogenes*, *Salmonella*, or STEC were enumerated using Modified Oxford, XLD, or Sorbitol MacConkey agar base, respectively, with thin layer overlay of nonselective media to enhance recovery of injured cells. Each study was replicated twice.

Results: Inactivation rates for STEC were similar to *Salmonella* at all four temperatures, and were comparable to or less than the times reported in Appendix A. In contrast, *L. monocytogenes* showed greater thermotolerance than *Salmonella*and STEC under all conditions. For example, > 5-log reduction of *Salmonella* and STEC was achieved at 71.1 °C instantaneous, whereas *L. monocytogenes* was inactivated within 15 seconds. At 65.6 °C, STEC and *Salmonella* populations decreased 5 log within 60 seconds, whereas ca. 5 minutes was required to inactivate 5 log *L. monocytogenes*.

Significance: Results support Appendix A as an acceptable tool for *Salmonella* and STEC lethality and provide new thermal processing guidance to appropriately address pathogenic bacteria in RTE roast beef. Additional studies will be completed in ham and turkey to confirm inactivation.

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P3-25 Rapid Quantitative and Qualitative Assessment of Minced Pork Meat Spoilage Using Fourier Transform Infrared (FTIR) Spectroscopy Data

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Introduction: FTIR spectroscopy has substantial potential as a quantitative control method in the food industry and it has been successfully employed so far to tackle authentication and adulteration problems in various foods.

Purpose: The aim of this work was to investigate the feasibility of FTIR spectroscopy to quantify biochemical changes in fresh, minced pork meat in the attempt to monitor spoilage.

Methods: Minced pork meat was stored under modified atmospheres (40% CO_2:30% O_2:30% N_2) at 0, 5, 10, and 15 °C, and samples were withdrawn to undergo microbiological, sensory and FTIR analysis. Partial least squares (PLS) models were constructed to correlate spectral data with meat spoilage in order to classify meat in 3 predefined sensory classes (fresh, semi fresh and spoiled). Moreover, PLS regression (PLS-R) models were developed to correlate the population dynamics of the microbial association with FTIR spectral data. The performance of the models was based on graphical plots and statistical indices.

Results: Results demonstrated good performance in classifying meat samples in the predefined sensory classes with overall correct classification of almost 81.8% (sensitivity within the classes: 87.5%, 55.3%, and 97.1% for fresh, semi-fresh, and spoiled, respectively). For PLS-R models, the values of the bias factor were close to unity for all microbial groups, indicating no systematic bias, while the calculated values of the accuracy factor showed that the average deviation between predictions and observations was 6.0% and 5.9% for total viable counts and *Pseudomonas* spp. and 8.7% and 10.5% for *B. thermosphacta* and lactic acid bacteria, respectively. Finally, correlations within 0.75-0.83 were calculated for all microbial groups with the exception of *Pseudomonas* spp. where the respective value was lower (0.65).

Significance: FTIR could become an interesting tool to monitor minced pork spoilage through the measurement of biochemical changes occurring in the meat substrate.

The authors acknowledge the Symbiosis-EU (*www.symbiosis-eu.net*) project.

P3-26 Determination of Transfer of Methicillin-resistant *Staphylococcus aureus* from Retail Pork Products onto Food Contact Surfaces and the Potential for Consumer Exposure

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Introduction: Methicillin-resistant *Staphylococcus aureus* is of increasing concern due to its resistance to multiple antibiotics and its recent detection in retail meat products. Current scientific literature suggests that the amount of MRSA in pork products is low. However, the possible impact on food safety at the consumer level due to retail pork products contaminated with MRSA still needs to be elucidated.

Purpose: The aim of this study was to evaluate the potential risk of consumer exposure to MRSA from contaminated food contact surfaces and contaminated retail pork products.

Methods: Pork loins, bacon and pork sausage were inoculated with a four strain MRSA cocktail, swabbed for initial bacterial populations, vacuum packaged and stored for two weeks at 4 °C to simulate normal packaging and distribution. Polyethylene cutting boards, knives and pork skin were contaminated with the inoculated product lying on the surface for 5 minutes. Polyethylene cutting boards and knives were also contaminated by placing a 500 g lead donut on the product while it is dragged across the transfer surface. 5 cm x 5 cm areas were swabbed and bacterial populations of the inoculated pork products and contact surfaces were enumerated on Baird-Parker Agar and reported as log cfu/cm^2.

Results: All 6 pork loins inoculated with 10 CFU/ml MRSA cocktail and swabbed before storage yielded an average of 4.8 log CFU/cm^2. Post-storage swabs of transfer surfaces in contact with the contaminated loin were 3.9 log CFU/cm^2, 2.7 log CFU/cm^2 and 4.1 log CFU/cm^2 for the cutting board, knife and pork skin, respectively. Post-storage swabs of the pork skin after weighted exposure to the contaminated transfer surfaces yielded 3.3 log CFU/cm^2 and 3.2 log CFU/cm^2 for the cutting board and knife, respectively. Similar log CFU/cm^2 values were observed for the bacon and pork sausage samples.

Significance: These results suggest that there is a potential risk for consumer exposure to low cell concentrations (10 CFU/cm^2) of MRSA on pork products.

P3-27 First Isolation of Shiga Toxin-producing *Escherichia coli* O157:H7 in Ground Beef at Retail Market in Sao Paulo City, Brazil

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Introduction: Shiga toxin-producing *E. coli* (STEC) serotype O157:H7 is still considered the main serotype of this group involved in outbreaks of foodborne illnesses. Despite the importance of ground beef as a vehicle for STEC, little is known about their presence in this food sold in Sao Paulo city, SP, Brazil.

Purpose: This study aimed at investigating the presence of STEC in ground beef at retail level in Sao Paulo city, Brazil, characterizing the virulence factors stx_1, stx_2, *eae* and *ehx* and identifying *E. coli* O157:H7 isolates using the genes *uid*, rfb_{O157} and $fliC_{H7}$.

Methods: 248 samples were acquired at the different districts of Sao Paulo city. Microbiological tests for the detection of STEC were performed following the ISO 16654 methodology for the detection of *E. coli* O157 serogroup. Suspected colonies were evaluated for the presence of target genes.

Results: One sample (0.4%) of ground beef showed the presence of STEC O157:H7. This isolate carried all investigated virulence factors: stx_1, stx_2, *eae* and *ehx*. Besides the presence of *uid*, rfb_{O157} and $fliC_{H7}$, serotype O157:H7 was also serologically confirmed at Institute Adolfo Lutz. Moreover, the expression of *stx* genes in Vero cells was also observed.

Significance: This is the first report on the isolation of STEC O157:H7 from ground beef in Brazil showing not only all virulence factors but also expressing Shiga toxin. Therefore, the ingestion of undercooked ground beef can be a risk.

P3-28 Through-chain Enumeration and Genotyping of *Campylobacter* spp. in Broiler Chicken Production

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Introduction: *Campylobacter jejuni* and *C. coli* are the leading causes of human foodborne bacterial gastroenteritis worldwide and frequently colonize poultry. These organisms become highly prevalent in broiler flocks after 2-3 weeks of age (~ 65% prevalence and 6-8 log CFU/g feces) and are widely disseminated during processing (~85% post-chill positive, mean 0.70 log CFU/cm^2). However, there are few studies which have attempted to quantify *Campylobacter* spp. in the same flock from primary production to processing, as well as tracking the genetic diversity of isolates.

Purpose: To determine (i) the time and levels of *Campylobacter* colonization in broiler chickens, (ii) the effect of commercial processing steps on the reduction of *Campylobacter* spp., and (iii) the sources and genetic diversity of the organism using rep-PCR fingerprinting.

Methods: Using a semi-quantitative technique, levels of *Campylobacter* were determined in broiler flocks from placement to processing. Feces (*n* = 350) and environmental samples from four free-range and conventional farms in the Sydney basin were analyzed at weekly intervals. Various samples (*n* = 180) from three of the flocks were collected throughout processing, from delivery to post-chill, and analyzed. Confirmed *C. jejuni* and *C. coli* isolates (*n* = 270) were selected and typed using rep-PCR for cluster analysis and classified against a library containing strains previously characterized using *fla*A-RFLP and MLST.

Results: Colonization of the broilers occurred as early as 7 days, with 100% infection of the flock between 2-3 weeks of age (mean 6 log CFU/g faeces). A significant 5 log reduction in *Campylobacter* levels was observed post-spin chill. There was broad genetic diversity among *Campylobacter* species, with seven clusters of *C. jejuni* and five clusters of *C. coli.*

Significance: Determination of the sources and time of colonization, in addition to baseline data, allows for the development of targeted intervention strategies for the control of *Campylobacter* spp. in broiler chickens.

P3-29 Cross-laboratory Comparative Study of the Impact of Experimental and Regression Methodologies on *Salmonella* Thermal Inactivation Parameters

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Introduction: Thermal inactivation studies often are aimed at testing the effects of various product factors on the inactivation rate of pathogens. However, the lack of standardized methods causes significant difficulties when trying to compare results across studies, and also limits the utility of merged data sets for meta-analyses or improved inactivation models.

Purpose: Therefore, the objective was to conduct a comparative study across two laboratories to determine whether experimental methodologies for isothermal inactivation tests, or data processing methods, significantly affect resulting inactivation parameters.

Methods: Using the same batch of irradiated ground beef (2.3% fat) and an identical 8-serovar *Salmonella* cocktail, two separate laboratory groups (MSU and USDA) each performed two different isothermal inactivation trials (reps ≥ 2) at 60 °C, each using two methods previously published by each group. The mean initial inoculation level across all trials was 7.2 log CFU/g. The raw data were then pooled and analyzed independently by each group, with MSU computing D-values using linear regression of the log CFU/g data, and USDA computing D-values from the rate parameter from regression of the same data using DMFit. The net result was a 2x2x2 study, with two laboratories, two experimental methodologies, and two analysis/regression methods.

Results: In pairwise comparisons of the survivor data using analysis of covariance, the *P* values for intra-laboratory results (across methods) were 0.07 and 0.01, and for intra-method results (across laboratories) were 0.07 and 0.45. When comparing the D-values determined using the two different regression methods by the respective groups, the differences were 3-6% or 21-40% when analyzing data generated by the two respective experimental methodologies, with D-values ranging from 0.88 to 2.2 min.

Significance: Overall, the results indicate that experimental methodologies and data processing methods can significantly impact reported inactivation parameters across multiple laboratories, even when using identical cultures and food materials, supporting the importance of fully disclosing methodology details and considering standardization of methodologies in the field.

P3-30 Inhibition of *Listeria monocytogenes* and *Leuconostoc mesenteroides* in an Uncured Deli-style Turkey Breast Using Clean Label Antimicrobials

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Introduction: Antimicrobials are an important ingredient for food safety. Recent interest in natural and organic meat and poultry processing have resulted in the need to identify and validate clean label antimicrobials in products with different compositional factors.

Purpose: To determine the effect of moisture, pH, and cultured sugar-vinegar blend combinations to inhibit *Listeria monocytogenes* or *Leuconostoc mesenteroides* in uncured turkey stored at 4 °C for 16 weeks.

Methods: Twenty uncured deli-style turkey breast treatments were generated using a central composite design for three variables, moisture (53.7-86.3%), pH (5.6-6.6) and cultured sugar-vinegar blend (0-5.79%) with five levels for each variable (center point treatment replicated 5 times). Sliced cooked products were surface inoculated with 3 log CFU/g of *L. monocytogenes* or *L. mesenteroides* (5-strain mix), vacuum packaged (100 g/package), and stored at 4 °C for up to 16 weeks. Triplicate samples per treatment were assayed at 0, 1, 2, 4, 6, 8, 12, 14, and 16 weeks by enumerating on Modified Oxford or APT agars. Finished products were analyzed for moisture, pH, NaCl and a_{w}.

Results: Treatments in this study had a greater inhibitory effect on *L. monocytogenes* than *L. mesenteroides*. The replicates of the center point (70% moisture, 3.75% antimicrobial and pH of 6.1) supported <1-log increase of *L. monocytogenes*at 16 weeks whereas all replications supported >2-log increase of *L. mesenteroides* at 2 weeks. Five of the 20 treatments supported >1-log increase of *L. monocytogenes* during 16 weeks of storage; the pH was ≥6.4 in four of those five treatments. Sixteen of 20 treatments supported growth of *L. mesenteroides* within 2 weeks. *L. mesenteroides* was inhibited in three treatments with ≥5.0% cultured sugar-vinegar blend.

Significance: A predictive model based on the interaction between moisture, pH and cultured sugar-vinegar blend will be developed to help meat processors identify formulations to inhibit *L. monocytogenes* and spoilage microbes.

P3-31 A Biotracing Model of *Salmonella* in the Pork Production Chain

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Introduction: In biotracing systems, downstream chain information and model-based approaches are used to trace the sources of microbial contamination in a food chain. Such more systematic and hence faster approaches can be used to trace the sources of *Salmonella* contamination in the pork slaughter chain.

Purpose: To trace the sources of contamination for individual *Salmonella*-positive carcasses at the end of the slaughter line.

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Methods: The model is a Bayesian Belief Network, in which *Salmonella* concentration information at different locations in the slaughterhouse are used in combination with prior knowledge about the dynamics of *Salmonella* through the slaughter line. Data, collected in a Dutch slaughterhouse, were used to specify prior beliefs about the model inputs and to iteratively refine the distributions of the parameters in the model so that it optimally describes that specific slaughterhouse.

Results: The model results indicate that house flora on or in the carcass splitter was the causative source of contamination for many carcasses, especially for those that carried contamination on the cutting side. Furthermore, the model indicates that the parameter values of the model may be subject to temporal variation and can be used as a tool to provide estimates of such trends.

Significance: The model shows the concept of biotracing, gives insight in the dynamics of *Salmonella* in the slaughter line and indicates the sites in the line where collecting data is most effective for biotracing. This biotracing model is implemented as an interactive computer application, which can be seen as a step in the process towards an operational biotracing system, by which a stakeholder can initiate immediate responses to *Salmonella* contamination and other hazards imposed in the pork slaughterhouse.

P3-32 The Use of Zero-valent Iron Filtration to Reduce *Escherichia coli* and *Listeria innocua* in Irrigation Water

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Introduction: Irrigation water can be a source of contamination in outbreaks associated with produce. Zero-valent iron (ZVI) filtration has been effective in *E. coli* O157:H12 in irrigation water, but has not been evaluated against *Listeria*spp.

Purpose: To 1) determine effectiveness of ZVI filters against *L. innocua* and *E. coli* O157:H12 in contaminated irrigation water, and 2) determine residual ZVI antimicrobial activity in filtered water inoculated with *E. coli*.

Methods: HydrAid biosand filters were built containing gravel and coarse sand. Two columns were then modified to contain fine sand only (S), and two columns contained a mixture of ZVI and fine sand at a 1:1 ratio. *L. innocua* and *E. coli* O157:H12 were cultured separately in bovine manure slurry and then inoculated in water at between 6 and 8 log CFU/100 ml, respectively, on three separate occassions. Contaminated water was filtered through either S or ZVI columns and collected for analysis of bacterial populations. To determine residual antimicrobial activity of ZVI, water filtered through S- or ZVI-columns was inoculated with populations of three *E. coli* strains at populations between 6.5–8 log CFU/ml.

Results: ZVI columns reduced *L. innocua* and *E. coli* O157:H12 populations in water by 6.5–6.9 and 6.8–7.4 log CFU/100ml, respectively. S columns were less effective, reducing *L. innocua* and *E. coli* by 0.8–2.2 and 0.8 –2.7 log CFU/100ml, respectively. *E. coli* O157 strains declined more rapidly (6.5–8 log CFU/ml) in ZVI-filtered water than in S-filtered water (2.9-3.3 log CFU/ml), over 8 days, while non-O157 *E. coli* declined less rapidly in ZVI- and S-filtered compared to O157 strains.

Significance: ZVI columns were effective in reducing *L. innocua* and *E. coli* populations in irrigation water, and showed residual activity against *E. coli* populations after filtration. ZVI filtration may provide more effective mitigation treatment for irrigation water compared to sand filtration.

P3-33 Microbiological Survey of Domestically-produced Sprouts Available at Retail in Canada

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Introduction: Since 1990, sprouts have been linked to 38 documented outbreaks in North America, and, more recently, were implicated in French and German outbreaks in which a novel *Escherichia coli* pathovar caused spectacular levels of morbidity and mortality. Despite well-recognized food safety issues with sprout production, sprouts remain popular and are frequently consumed without cooking.

Purpose: Examine the microbiological quality and safety of retail sprouts in Canada, and to determine whether antimicrobial resistance (AMR) is a concern in retail sprouts.

Methods: Domestically produced sprouts (n=44) were obtained from five cities across Canada. Total microbial counts, coliforms and *E. coli* were determined by standard methods. In addition, *E. coli* O157:H7 and *Salmonella* were detected using Reveal kits and conventional testing methods, respectively. Enrichment was used to recover enterococci, with all isolates screened for AMR using a panel of 15 antimicrobials.

Results: Overall, total microbial loads were high, with 80% of samples possessing ≥ $2x10^8$ CFU/g. Coliforms were detected in 50% of all samples, with one sample (2.3%) positive for generic *E. coli*. Neither *E. coli* O157:H7 nor *Salmonella*were detected. Enterococci were recovered from 93% of samples; 7% of the isolates were *En. faecalis*, 24% *En. faecium* and 68% *Enterococcus* spp. AMR examination revealed resistance to clinically relevant antibiotics, including kanamycin, nitrofurantoin, penicillin, and quinupristin.

Significance: Overall, the observed levels of total bacterial and coliform levels were similar to previous reports. However, the observed prevalence of enterococci and associated AMR represent an ill-considered transmission source of antimicrobial resistance.

P3-34 Plant Defense Response to *Escherichia coli* O157:H7 Cell Surface Structures Influences Survival of the Enteric Pathogen on Plant Surfaces

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Introduction: Bacterial surface components such as curli, flagella, lipopolysaccharides, and exopolysaccharides have been shown to play an important role in the interactions between bacteria and plant surfaces. It has been suggested that induction of plant defense responses by human enteric pathogens may influence their colonization on the plant.

Purpose: The objectives of this study were to investigate how *E. coli* O157:H7 cell surface structures influence the plant host defense responses and subsequently affects colonization or survival of the pathogen on plants.

Methods: *Arabidopsis thaliana* ecotype Columbia (Col-0) wild-type and *Arabidopsis BGL2-GUS* transgenic plants were dip inoculated with *E. coli* O157:H7 strain ATCC 43895 and strain 86-24, and isogenic mutants lacking specific cell surface structure(s) at a concentration of approximately 10^8 CFU/ml in water for 30 s. At 0, 1, 3, and 5 days post-challenge wild-type *Arabidopsis* plants were harvested and the populations were determined by plating the homogenates on TSA supplemented with appropriate antibiotics. At day 5 post inoculation GUS activities of *BGL2-GUS* transgenic plants were determined to monitor plant defense responses.

Results: On day 5 post inoculation, the population of *E. coli* O157:H7 43895 wild-type and flagella mutant on *Arabidopsis* wild-type plant was 5.05 and 6.53 CFU/g, respectively. Plants inoculated with the 43895 wild-type strain showed more than a 2-fold increase in GUS activity compared with the 43895 flagella mutant. Curli-deficient *E. coli* O157:H7 86-24 strain (6.83 CFU/g) highly colonized wild-type *Arabidopsis* plants compared with the curli-producing 86-24 wild-type strain (5.76 CFU/g). GUS activity of plants inoculated with the 86-24 curli mutant and 86-24 LPS mutant

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exhibited a 3-fold lower GUS activity compared with plants inoculated with the 86-24 wild-type strain.

Significance: This study demonstrated that differences in bacterial cell surface structure of *E. coli* O157:H7 significantly influenced colonization through triggering differential plant defense responses.

P3-35 Comparison of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) with O157:H7 for Chlorine Sensitivity and Transfer during Washing of Romaine Lettuce

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Introduction: In the past decade, *E. coli* O157:H7 has caused multiple outbreaks of foodborne illness associated with leafy greens. Illnesses linked to non-O157 STEC appear to be on rise in the United States and worldwide, indicating that these strains have become significant threats.

Purpose: The aim of this study is to compare ten different serotypes of non-O157 STEC with O157:H7 in growth kinetics, chlorine sensitivity and the extent of transfer during lettuce washing, to provide information for preventing pathogen transfer in post-harvest processes.

Methods: The characterization of growth kinetics was conducted by using the Bioscreen C automated growth curve analysis system. Chlorine sensitivity was evaluated by analyzing the lag phase extension of growth in the presence of a sub-lethal concentration of chlorine (0.15 ppm), compared to no-chlorine controls. Pathogen transfer during washing was assessed in lettuce washing experiments. After inoculation of 10^6 CFU cells onto 3 pieces of lettuce leaf (0.5 g), the lettuce was washed in 50 ml cold water with uninoculated lettuce strips (0.5 g) for 1 min. Populations of the *E. coli* remaining on the inoculated leaves, in the wash water and transferred to the uninoculated leaves were enumerated by plate counts on selective agar.

Results: Thirty non-O157 STEC strains (including the USDA defined "Big Six", O104:H4 and three other O serotypes) showed growth kinetics similar to that of O157:H7. Doubling time was 25 to 30 min, lag phase duration was 3 hrs and maximum population was 10^9 CFU/ml, approximately. With only one strain in O111 group and another strain in O121 group showing less chlorine resistance, no significant difference in chlorine sensitivity was observed for the other "Big Six" non-O157 STEC strains and O104:H4 group when compared with that of O157:H7. On the other hand, O29, O73 and O78 strains exhibited higher chlorine sensitivity.

Preliminary cross-contamination experiments using the O104:H4 isolate from the 2011 German sprout outbreak and O157:H7 isolate from the 2006 US spinach outbreak showed similar transfer efficiencies by the two strains during lettuce washing. Log reduction from washed lettuce was approximately 2.3; log transferred from the lettuce to water was approximately 3.8 after 1 min, and to the uninoculated leaves was 2.8.

Significance: The information generated by this study will be useful to assess pathogen risk and to determine effective sanitization procedures for preventing STEC cross-contamination during washing of leafy greens.

P3-36 Microbial Survey of Surface Water Used for Fresh Produce Crop Irrigation in Pennsylvania

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Introduction: Foodborne illness outbreaks linked to contaminated irrigation water from surface sources has created a need for improved farm food safety practices. Although there are no national legally enforced standards for microbial safety of irrigation water for growing produce crops, several government and commodity groups have developed their own surface water guidelines.

Purpose: The purpose of this study was to survey microbial indicators and select human pathogens in surface waters used for irrigation of fresh produce crops in Pennsylvania, compare these levels to current surface water guidelines, and determine if indicator microorganisms reliably predict the presence of pathogens.

Methods: Over the course of 2 years, 153 1-liter samples were collected from 39 farms at 3 times during the growing season. Data were collected for 6 physical attributes (conductivity, turbidity, pH, dissolved oxygen, air and water temperature), 14 environmental characteristics (including source type, precipitation 0 and 3 days before sampling, adjacent animal activity, degree of sunlight exposure), 5 indicator organisms (*Enterobacteriaceae*, coliforms, fecal coliforms, *E. coli*, and enterococci) and 2 pathogens (*E. coli* O157:H7 and *Salmonella*).

Results: Among all samples collected, 61% exceeded EPA recreational water and California leafy greens surface water limits for *E. coli* (126 CFU/100ml), 67% exceeded GlobalGap limits for fecal coliforms (1000 CFU/100ml), and 51% exceeded EPA standards for enterococci (33 CFU/100ml). *E. coli* O157:H7 and *Salmonella* were not detected in any samples using two different plating techniques. Statistical analysis of combined 2-year data showed significant ($P < 0.05$) effects of certain physical attributes and environmental characteristics on *E. coli,* coliform, *Enterobacteriaceae,* and enterococci although significance of individual effects varied between years.

Significance: Microbial indicator levels varied greatly between farms and sampling times. Despite frequent failures to meet established water standards, indicator microorganisms did not reliably predict the presence of human pathogens as determined by plating techniques. The utility of microbial indicators to predict farm food safety risks must therefore be further examined.

P3-37 Survival of Foodborne Pathogens on Cilantro Plants after Transfer via Wet- and Dry-Inoculation Methods

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Introduction: Preharvest produce may be contaminated with foodborne pathogens in both wet and dry matrices; how the mode of introduction impacts survival is unknown.

Purpose: To compare survival of foodborne pathogens on wet- and dry-inoculated cilantro plants in the field and in an environmental growth chamber.

Methods: Mature cilantro plants were inoculated with rifampicin-resistant attenuated strains of *Escherichia coli* O157:H7 ATCC 700728 (commercial field Salinas, California and laboratory growth chamber) and *Salmonella* Typhimurium (laboratory only). Bacteria were applied to the plants (4 to 8 log CFU/g) via spraying an aqueous solution or dusting freeze-dried bacterial powders mixed with chalk. Survival for up to 10 h in the field or 48 h in the laboratory (23 °C, 30% and 80% RH) was compared. To recover inoculated bacteria, plants were added to 0.1% peptone and stomached, and then samples were plated onto TSA or filtered onto CHROMagar O157 or CHROMagar *Salmonella*, all containing rifampicin.

Results: In the field, *E. coli* O157:H7 populations declined by 3 log after 10 h on cilantro plants inoculated by either method. These declines were similar to those observed a growth chamber set to 30% RH (2 and 3 log reduction after 6 h for wet and dry inoculation, respectively). *Salmonella* populations changed by < 1 log for wet and dry inoculation methods under the same conditions. In contrast, at 80% RH, *E. coli* O157:H7 and *Salmonella* populations on cilantro were consistent over 48 h when inoculated via either wet or dry methods.

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Significance: Inoculation method did not affect survival of *E. coli* O157:H7 or *Salmonella* on cilantro plants. Low RH better mimics survival of foodborne pathogens under field conditions in Salinas, CA.

P3-38 Influence of Constituents of Water, Soil or Manure on Colonization of *Escherichia coli* O157:H7 on Plant by Differential Induction of Plant Defense

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Introduction: Contaminated irrigation water, soil, or manure has been considered potential contamination sources of fresh produce on the farm. Physiological changes may occur in *E. coli* O157:H7 growing in or exposed to water, soil, or manure influencing plant colonization by differential induction of plant defense response.

Purpose: The objective of this study was to determine influence of constituents of water, soil, or manure on colonization of *E. coli* O157:H7 on plant tissue by plant defense response.

Methods: *Arabidopsis thaliana* ecotype Columbia (Col-0) wild-type and *Arabidopsis BGL2-GUS* transgenic plants were dip inoculated with *E. coli* O157:H7 ATCC 43895, grown or exposed to water, soil or manure, at a concentration of approximately 10^8 CFU/ml in water for 30 s. At 0, 1, 3, and 5 days post challenge wild-type *Arabidopsis* plants were harvested and the populations were determined by plating the homogenates on TSA supplemented with appropriate antibiotics. At day 5 post inoculation, GUS activities of *BGL2-GUS* transgenic plants were determined to monitor plant defense responses.

Results: On day 5 post inoculation, the population of *E. coli* O157:H7 grown in LB alone, LB supplemented with soil or manure on *Arabidopsis* wild-type plant was 5.06, 5.51, and 6.36 CFU/g, respectively. *E. coli* O157:H7 exposed to water, soil, or manure (6.98, 6.76, and 7.10 CFU/g, respectively) showed greater colonization on the wild-type plant compared to control (non-exposed) *E. coli* O157:H7 (5.06 CFU/g). *E. coli* O157:H7 cultured in LB exhibited 2-fold greater GUS activity compared with cells grown in LB with soil or manure extracts. *E. coli* O157:H7 exposed to water or manure showed 3-fold less GUS activity compared with non-exposed cells.

Significance: This study demonstrated that physiological changes of *E. coli* O157:H7 occurring following exposure to water, soil, or manure may influence plant defense response, and consequently impact colonization of the plant.

P3-39 Examination of Irrigation Water as an On-farm Bacterial Reservoir and Potential Contamination Route for In-field Leafy Greens

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Introduction: In the production of produce, irrigation water quality has been a primary concern for the produce industry. Despite this, the causal relationship between irrigation water quality and produce safety is not well understood.

Purpose: To examine how poor and high quality irrigation water influences the microbiological quality of in-field leafy greens grown in a conventional production system.

Methods: Pre-harvest plant (n=279) and irrigation water samples from two sources (municipal water [n=9] and ditch water [n=19]) were collected from a conventional farm production system in British Columbia (BC), Canada, weekly between August and October 2011. Levels of coliforms and *Escherichia coli* were determined using 3M Petri-Films. Recovered *E. coli* isolates were subjected to antibiogram typing and phylogenetic multiplex PCR analysis.

Results: Levels of coliforms on leafy greens ranged from 0.7 to 2.0 log CFU/g, with an average of 1.2 ± 0.1 log CFU/g. No *E. coli* isolates were recovered from leafy greens irrigated with municipal water whilst one sample of 149 (0.7%) irrigated with ditch water tested positive. While no coliforms were detected in municipally treated water, coliforms and *E. coli* were detected in 19/19 ditch water samples, with average levels of coliforms and *E. coli* of 4.1 ± 0.2 log CFU/100ml and 2.7 ± 0.3 log CFU/100ml observed, respectively. *E. coli* isolates belonging to all four phylogroups (A, B1, B2, D) were detected in ditch water.

Significance: Regardless of whether in-field produce was irrigated with ditch or municipal-quality water, the levels and frequency of coliforms and *E. coli* recovery was low. Further, the low incidence of *E. coli* observed in produce irrigated with poor quality water suggests undefined factors may impair *E. coli*'s ability to survive following transition from an aqueous milieu to the plant biosphere.

P3-40 Fate of Protozoan Oocysts (*Eimeria papillata*) on Lettuce in Field Plots

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Introduction: Fresh produce is a recognized vehicle for the transmission of illness caused by protozoa such as *Cryptosporidium*, *Cyclospora* and *Giardia*. The hardiness of protozoan oocysts in the laboratory has been documented but little is known about their fate on field crops.

Purpose: The objective of this work was to measure time-dependant changes in populations of oocysts from the surrogate species *Eimeria papillata* following their inoculation onto field lettuce and to use the data in the development of a predictive mathematical model.

Methods: Known concentrations of *E. papillata* sporulated oocysts in water were sprayed onto four-week-old Romaine lettuce plants in field plots at two locations (BC and NS) in three separate trials. Lettuce samples were collected on 0, 1, 2, 3, and 4 weeks post-inoculation, and oocysts eluted and enumerated using standard parasitological methods. Changes in log numbers were plotted against time, non-linear regression was performed and data were fitted with a Weibull decline function.

Results: Oocyst concentrations declined from 2 log CFU/g to <1 log CFU/g over four weeks but there was between-trial variation in the time required for the first log reduction and the Weibull shape parameter. A bootstrapping technique was applied to develop cumulative distributions used to simulate additional datasets (25), which then were fitted with the Weibull function to derive a predictive model. Data points generally fell within the 95% prediction limits defined by the model, and in a few cases exceed this range. Hence the model adequately predicted the fate of *E. papillata* oocysts in field lettuce.

Significance: The proposed model will be useful for the development of quantitative microbial risk assessments for protozoan oocysts in leafy vegetable field crops.

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P3-41 Influence of Mycorrhizal Fungi (*Glomus intraradices*) on Survival of *Salmonella* and *Escherichia coli* O157:H7 in Soil and Translocation into Romaine Lettuce Roots and Shoot

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Introduction: Modern agriculture practices disrupt the natural symbiotic relationship that arbuscular mycorrhizal (AM) fungi have with most vegetable plants, which may affect translocation of human pathogens into the plant and/or survival in the soil. AM-fungi are frequently utilized in organic farming to improve plant productivity, soil quality and to increase drought resistance.

Purpose: To determine the influence of AM-fungi (AMF) on survival of *Salmonella* and *E. coli* O157:H7 (EHEC) in soil and translocation into plant roots and shoot.

Methods: Six-week-old Romaine lettuce plants (with or without AMF [*Glomus intraradices*]) were inoculated (8 ml per plant) with composite suspensions of *Salmonella* or EHEC (8 log CFU/plant). Samples were taken one day following inoculation and then weekly up to 22 days. Samples were plated on XLT4PN and tcSMACPN, respectively. Pre-enrichments were made in TSBPN, while secondary enrichments were in TTPN for *Salmonella* and a novel TSBselect broth for EHEC.

Results: *Salmonella* and EHEC both internalized Romaine roots and shoots for up to 22 days following inoculation. Overall, there was little difference in pathogen populations between AMF+ and AMF- roots, shoots, and soil. This is in contrast to results attained from previous studies using a leek model. *Salmonella* populations were higher than EHEC $P < 0.05$) for 63% of all root and shoot samples; while final populations of the two pathogens in the soil at day 22 were ca. 4.3 and 1.2 log CFU/g, respectively. Selective enrichment samples (96%) for plant tissue were positive for *Salmonella* on day 22, while only 29% of EHEC enrichments were positive. Soil samples (100%) were positive for *Salmonella* by selective enrichment, and 83% were positive for EHEC by day 22.

Significance: These results are the first to suggest that AM-fungi do not influence the internalization of pathogens in Romaine seedlings, which may provide some assurance of the safe application of AMF in modern agricultural practices.

P3-42 Effectiveness of UV Light as a Means to Reduce *Salmonella* Contamination on Tomatoes

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Developing Scientist Competitor

Introduction: *Salmonella*-contaminated fresh produce has been linked to multiple foodborne illness outbreaks. Fresh produce receives little microbial intervention other than washing. An alternative strategy to reduce the pathogenic bacterial levels on fresh produce is to use ultraviolet-C light at a wavelength of 200-280 nm. However, repair of bacterial injury by photoreactivation with visible light is a concern.

Purpose: This study evaluated the effectiveness of UV-C light (254 nm) in reducing *Salmonella* contamination on tomatoes under different UV-C treatments and possible photoreactivation of injured *Salmonella* post-UV-C treatments with visible light.

Methods: Tomatoes were spot inoculated with approximately 5 log cfu/ tomato with rifampicin-resistant *Salmonella* and air-dried before exposing to UV-C light at different doses (0, 1, 2, 4, and 6 mJ/cm^2) at a distance of 60 cm. After the UV-C treatments, the tomatoes were exposed to fluorescent light at 0, 0.85, 4.26, and 20.42 J/cm^2. Treated tomatoes were rinsed with 20 ml of 0.1% peptone and spiral-plated onto tryptic soy agar plus rifampicin (TSAR). Plates were incubated at 37 °C for 24 h before enumerating surviving *Salmonella*.

Results: UV-C dosages of 1 and 2 mJ/cm^2 reduced the *Salmonella* population by 2 logs. *Salmonella* populations were reduced by >2.56 logs when the tomatoes were treated with 4 and 6 mJ/cm^2. There was no evidence of injury repair by photoreactivation by visible light treatments.

Significance: Use of UV-C light may be a promising means to reduce *Salmonella* contamination on fresh tomatoes.

P3-43 Survival of Non-pathogenic *Escherichia coli* and *Escherichia coli* O157:H7 in Delmarva Field Plots Amended with Animal Manure

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Introduction: Land application of raw animal manure to enhance soil productivity may pose a food safety risk from pathogenic microorganisms that survive and contact fresh produce grown on the soils. The presence of pathogens in fresh produce has resulted in several outbreaks of human gastroenteritis that have been linked to the consumption of contaminated fresh produce.

Purpose: Evaluate and compare the survival and persistence of a multi-strain cocktail of non-pathogenic *E. coli* (*Ec*) and attenuated *E. coli* O157:H7 (attO157) as influenced by the application of poultry litter (PL) or dairy manure (DM) on field plots in the Delmarva peninsula.

Methods: A field experiment comprising eight treatments with four replications each was conducted in Othello soil at the University of Maryland Eastern Shore Ag Research Farm. Soil plots were individually amended with or without PL or DM in late fall. Individual plots, except controls, received spray inoculum at either low, 5×10^6 CFU/m^2, or high, 5×10^8 CFU/m^2, cell densities; inocula contained three rifampicin-resistant (Rif^R) strains of *Ec* and two Rif^R strains of attO157 grown in dairy manure extract. Soil samples collected for 30 days post-inoculation were analyzed for viable *E. coli* by direct plating and/or mini-MPN.

Results: Survival of *Ec* and attO157 populations declined more rapidly in DM compared to PL treatments. By 30 days, at high inoculum densities, *Ec* counts on PL and DM declined to 4.6 and 2.43 log CFU/g, respectively, and attO157 counts declined to 3.83 and 0.46 log CFU/g, respectively. No *Ec* or attO157 were found in control soil.

Significance: Manure type influenced survival of *E. coli* in soil, with attO157 declining more rapidly than *Ec*. These findings should be taken into consideration when setting guidelines for fresh produce safety relative to PL and DM application to soil.

P3-44 Internalization of *Salmonella* Typhimurium in Hydroponically Grown Mung Bean Sprouts with the Events of Water Contamination

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Introduction: Mung bean sprouts, a popular item typically found in Asian cuisine, are consumed raw or minimally cooked and are often at high risk for containing foodborne pathogens such as *Salmonella*. Most previous studies about bean sprouts were focused on contaminated seeds and the fate of pathogens. However, if irrigation water contains pathogens, there is a chance for the pathogen to contaminate the sprout. Internalized pathogens especially pose high risk since conventional sanitization is ineffective to inactivate them.

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Purpose: The goal of this study is to understand the potential of internalization of *Salmonella* in mung bean sprouts with the presence of the bacteria in irrigation water. Our hypothesis is that the extent of *Salmonella* internalization is associated with the level of contamination in water.

Methods: Mung bean sprouts were grown hydroponically and were given GFP-tagged *Salmonella* Typhimurium (ca. 10^9 CFU/ml) after germination through maturity. One set received the contaminated water daily for five days while the other five sets received contaminated water only one of the five days. Samples were harvested daily and their surfaces were disinfected using ethanol and $AgNO_3$. Internalized *S.* Typhimurium was counted using LB agar with 0.1% ampicillin. Green fluorescent colonies were counted after incubating for ~24 hrs at 37 °C.

Results: The internalization of *Salmonella* was observed in all contamination events at the level of 2.0-5.1 log CFU/g sprout. Continuous watering with contaminated water during the entire period generated statistically higher levels of*Salmonella* internalization than those five sets that received contaminated water only for a single day ($P < 0.05$).

Significance: The presence of *Salmonella* in water is subject for the bean sprouts to become internalized with the pathogen at high enough levels to result in illness if ingested. Therefore, proper procedures in regards to using and maintaining clean water must be followed throughout the bean sprouts production chain.

P3-45 Microbiological Quality of Imported Produce Available at Retail Across Canada

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Introduction: Rising consumption trends of fresh, minimally processed produce in Canada has led to increased reliance on imported produce. Concern regarding potential risks associated with produce production systems, particularly in the developed world, have recently been expressed.

Purpose: Our goal was to examine the microbiological quality of imported vegetables available in Canada.

Methods: Fresh imported produce (61 herb, 20 spinach, and 25 leafy greens) from five cities across Canada were microbiologically tested for quality (total bacterial count, coliforms, enterococci, *Escherichia coli*) and the presence of*E. coli* O157:H7 and *Salmonella* spp. Recovered enterococci were screened for antimicrobial resistance (AMR). The mobility of R-plasmids in enterococci was examined through transformation into *Staphylococcus aureus*.

Results: No pathogenic bacteria were recovered. With the exception of one sample, total microbial loads were >4 log CFU/g. Overall, coliforms were detected in 45.3% of samples, enterococci in 55.7%, and *E. coli* in 3.8%. Produce originating from Columbia had the highest recovery of *E. coli* (11.1%). Of enterococci recovered, 33.9% were *Enterococcus faecium* and 16.4% were *En. faecalis*; in Mexican produce, these were recovered at a rate of 44.3% and 9.8%, respectively. Enterococci were observed to be resistant to antimicrobials used in animal feed, including bacitracin, tetracycline, and penicillin. Additionally, reduced susceptibility to clinically relevant antimicrobials (chloramphenicol, kanamycin, synercid) was also observed. *En. faecalis* R-plasmids possessing a *tet* gene were transferrable to *S. aureus* through transformation, but not conjugation.

Significance: Overall, a lack of pathogens and the observed incidence and levels of indicator organisms show imported produce to be of acceptable quality. However, the transmission of clinically relevant antimicrobials *via* imported produce warrant further investigation to ascertain risk to the consuming public.

P3-46 Levels of Microbial Contamination during the Production Chain of Tomatoes, Jalapeño Peppers and Melons in Northeast Mexico

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Introduction: Fresh produce items imported from Mexico including melons, tomatoes and jalapeño peppers, have been linked in the past to outbreaks of microbial disease. Little is known about produce contamination during the growing, harvesting and packaging processes.

Purpose: To determine the levels of microbial contamination of fruits and vegetables during the production chain in northeast Mexico.

Methods: In this study, 4 tomato farms, 7 jalapeño farms and 3 cantaloupe farms were screened. A total of 55 samples of soil, 89 samples of irrigation and source water, 161 samples of produce and 106 samples of hand rinse from farm workers were collected during the harvest season. Levels of coliforms, *Enterococcus*, and *E. coli* were analyzed using filtration methods, and commercial kits. *Salmonella* spp. and *E. coli* O157:H7, were assayed by BAM procedures.

Results: Statistically significantly ($P < 0.05$) higher levels of indicator bacteria, compared to soil and water samples, were found in hand rinse samples. In hand rinse samples, levels of coliforms ranged from 1×10^3 to > 1.5×10^4 CFU/ml, and levels of *Enterococcus* ranged from < 1 to > 5×10^4. Among produce, coliforms ranged from 4×10^2 to > 1.5×10^4 and < 1 to 5×10^5 CFU/ml for *Enterococcus*. Cantaloupes had significantly higher levels of indicator bacteria compared to tomatoes and jalapeños. Levels of *E. coli* were very low (< 5 CFU/ml) in water samples, however in hand rise samples levels of *E. coli* were significantly higher. *Salmonella* was found in one sample of melon and *E. coli* O157:H7 was found in one jalapeño pepper. Water and soil samples showed moderate levels of indicator organisms.

Significance: Cantaloupes exhibited higher contamination and hands are a potential source of contamination. Intervention approaches during the production chain are necessary to reduce the risk of infection in consumers.

P3-47 Impact of Inoculation Time (Evening or Morning) on *Escherichia coli* O157:H7 Survival on Pre-harvest Cilantro

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Introduction: Cool temperatures and high relative humidity (RH) are known to improve the survival of foodborne pathogens on preharvest produce plants.

Purpose: To compare the survival of *Escherichia coli* O157:H7 on cilantro plants inoculated in the evening (cooler temperature, higher RH) and morning.

Methods: Mature cilantro plants (4 and 6 weeks post seeding) were spray inoculated (7 log CFU/ml) with rifampicin-resistant attenuated *E. coli* O157:H7 ATCC 700728 in the evening (9 or 10 pm) or morning (7 am) in a commercial field (Salinas, California). Field temperatures, RH and bacterial survival were monitored until plants were 8 weeks post seeding. Harvested plants (n = 5 to 10) were stomached in 0.1% peptone. Sample preparations were plated onto tryptic soy and CHROM O157 agars; the remaining sample was enriched in tryptic soy broth. All media contained rifampicin.

Results: Survival of *E. coli* was consistently greater on plants inoculated in the evening than those inoculated in the morning during the first 2-days after inoculation. Populations decreased from 5 log CFU/g at the time of inoculation to 1 log CFU/g at 48 h. However, *E. coli* O157:H7 was

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isolated in 100% of plants by enrichment of 70 to 100 g samples of cilantro for up to 4 weeks after inoculation. Average field temperature and relative humidity (RH) during the 9 h after morning and evening inoculation were 14 °C and 93% RH, and 17 °C and 71% RH, respectively.

Significance: Environmental conditions at the time of inoculation may impact *E. coli* O157:H7 survival on cilantro plants for a short period after contamination.

P3-48 Indicator Methods to Evaluate Process Controls for Fresh Produce

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Introduction: The microbiological quality of leafy green processing water is currently evaluated through the use of bacterial indicators. Microbial monitoring of pre- or post-harvest waters may be a useful component of risk reduction strategies.

Purpose: The goals of this study were to compare traditional and automated quantification methods for microbial indicators, to reduce the labor and materials required for analysis, and improve process control.

Methods: Triplicate water samples (0.5 l) of end-of-day flume water (FW) with and without increased organic content and water drained from the centrifugal drier (CDW) were inoculated to contain approximately 5, 10 or 100 CFU/ml*Escherichia coli* 8739. Total organic carbon was measured for each sample. Samples and negative controls were analyzed by traditional plating methods and with the bioMerieux TEMPO automated MPN, in duplicate. MI agar was used for quantification of generic *E. coli* and total coliforms, violet red bile glucose agar was used for enumeration of *Enterobacteriaceae*, and total viable counts were determined by plating on trypticase soy agar. The plating results were compared to the results from the corresponding TEMPO assays.

Results: Uninoculated FW and CDW contained an average of 1.75 and 0.89 log CFU/ml total coliforms and 0.35 and 0.94 log CFU/ml *Enterobacteriaceae*, respectively. The average total viable counts for FW and CDW were 3.84 and 2.60 log CFU/ml, respectively. No *E. coli* was detected in either water type. Traditional and automated methods of microbial quantification were comparable ($P > 0.05$) with the exception of coliforms in inoculated CDW samples. The FW inoculated at 5 CFU/ml and 10 CFU/ml with increased organic content also showed significant differences between the coliform enumeration methods ($P < 0.05$).

Significance: Based on these findings, the TEMPO system may be an appropriate means for microbiological monitoring of produce process waters. This system could be used to rapidly assess the effectiveness of process controls or indicate a process failure.

P3-49 Microbial Cross-contamination of Tomatoes during Washing with a Peroxyacetic Acid-based Sanitizer in a Commercial Packinghouse

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Introduction: Post-harvest packing of tomatoes typically involves the use of a sanitizer in dump tank water during washing. However, sanitizer efficacy is known to decrease with increasing organic load.

Purpose: The goal of this study was to 1) assess the efficacy of a peroxyacetic acid-based sanitizer (Tsunami 100, Ecolab, St. Paul, MN) at 13, 52 and 72 ppm for reducing the microbial load on tomatoes and in dump tank water during commercial packing and 2) compare the efficacy of Tsunami 100 at 50 ppm in a pilot-scale packing line.

Methods: During three visits to one Michigan tomato packer, a series of tomato (~900 g), water (50 ml), equipment surface (100 cm^2) and brush samples (one bunch of bristles) were collected during 4 h of processing. All samples were appropriately neutralized, hand-rubbed/stomached, diluted and surface-plated on Standard Method Agar and acidified Potato Dextrose Agar with or without membrane filtration to enumerate mesophilic aerobic bacteria (MAB) and yeast/mold (YM), respectively. Water samples from the dump tank were also assessed for sanitizer concentration, oxidation/reduction potential, pH, temperature, chemical oxygen demand, and total solids. Additional unwashed tomatoes obtained from the same packer were submerged for 2 min in 50 ppm Tsunami 100 and then brush-washed using a pilot plant-scale roller conveyor. Tomato, water and brush samples were similarly collected and quantitatively analyzed for MAB and YM using standard plating methods.

Results: Initially and after 3 h of commercial processing, MAB populations decreased 0.29 and 0.17, 1.54 and -0.21, and 1.13 and 0.52 log on tomatoes using 13, 52 and 72 ppm Tsunami 100, respectively. Similarly, YM populations decreased 1.24 and 0.66, 1.71 and -0.11, and 1.65 and -0.5 log on tomatoes using 13, 52 and 72 ppm Tsunami 100, respectively. Microbial counts in the dump tank water tended to increase with organic load during processing. Both sets of brush rollers sampled before and after waxing were heavily contaminated. After pilot-scale processing, MAB and YM populations on tomatoes decreased 1.21 and 0.89 log CFU/g, respectively. The brush rollers were cross-contaminated by tomatoes with MAB and YM populations of 2.39 and 2.17 log CFU/bunch, respectively.

Significance: Based on inconsistencies in sanitizer concentration observed commercially, build-up of organic load in the dump tank water and the heavily contaminated brush rollers, more effective microbial intervention strategies are needed to minimize cross-contamination during tomato packing.

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P3-50 Survival of *Escherichia coli* O157:H7 on Raw Green Tomatoes during Transportation Temperature Abuse and Pathogen Transfer Efficacy between Tomatoes and Common Packaging Materials

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Introduction: Recently, *Escherichia coli* O157:H7, as well as other enteric pathogens, have been linked to foodborne outbreaks associated with produce.

Purpose: The risk associated with *Escherichia coli* O157:H7 survival on the surface and transfer between tomatoes and common packaging house materials were the purpose of this study.

Methods: Green, unwashed tomatoes, as well as squares of materials found in the packing house environment (stainless steel, vinyl belt, and HDPE, 3 x 3 in), were spot inoculated with a five-strain cocktail of rifampin-resistant *E. coli*O157:H7 and dried. Survival was monitored at 15 °C (tomatoes only), 25 °C (tomatoes and squares), and temperature abuse conditions (tomatoes only) for four days. Pathogen transfer from squares to tomatoes and vice versa were evaluated in wet, 90 min dry, and 24 h dry states either immediately or 24 h later. Pathogens were recovered in buffered peptone water, and plated on tryptic soy agar with 80 ppm rifampin for enumeration and percent positive calculation.

Results: *E. coli* O157:H7 numbers declined 1.4 log units after 90 min drying, and continued to decline at both 15 °C, 25 °C, and during temperature ramp (from 25 °C to 15 °C over 96 h), resulting in 2.4, 1.5, and 2.6 log unit reductions by day 4, respectively. The plate counts declined from an inoculation level of 6.1 log CFU/ml to below 1.0 log CFU/ml on average after four days at 25 °C for all squares. Wet transfers (zero drying time) yielded 100% positives on day 0 and day 1. Dry transfers (90 min and 24 h drying time) from tomatoes to squares showed

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higher transfer rates than the converse. Interestingly, vinyl belt picked up the most pathogen cells (90 min dry), resulting in 100% positive, followed by HDPE (66.7% positive) and stainless steel (55.6% positive).

Significance: Overall, *E. coli* O157:H7 did not survive well on the surfaces resulting in poor dry transfers under conditions tested.

P3-51 The Effect of Pesticides on the Growth and Survival of Foodborne Human Pathogens

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Introduction: Pesticides are widely used to control pests in the field production of fresh fruits and vegetables. Depending on the interaction between pesticides and human pathogens, pesticide sprays on fresh produce could be a source of contamination of human pathogens or an additional control for such contamination.

Purpose: The aim of this study was to evaluate the effect of pesticides on the growth and survival of the foodborne human pathogens *E. coli* O157:H7 and *Salmonella* Typhimurium.

Methods: Eight pesticides that are commonly used on leafy greens and/or tomatoes were tested at three concentrations, ranging from the lowest to highest as recommended in their applications. Each pesticide solution was prepared in sterile distilled water containing ca. 4 log CFU/ml of each pathogen individualy and incubated at 21 °C. The population of each human pathogen in pesticide solution was monitored up to 24 h.

Results: The fungicide/bactericide copper hydroxide (Kocide); and the insecticides Assail, Mustang and Ambush were significantly ($P < 0.05$) inhibitory to both pathogens in a concentration dependent manner. The fungicide chlorothalonil (Bravo), and the insecticides Beleaf and Intrepid did not show significant effect on both pathogens. However, the fungicide azoxystrobin (Quadris) showed a significant ($P < 0.05$) stimulatory growth effect only on *E. coli* O157:H7 after 6 h of incubation at 21 °C.

Significance: The findings of this study indicate that, depending on the specific pesticide, some may serve as a potential carrier of human pathogens to fresh produce if contaminated water is used in their preparation, whereas others could be an additional barrier to such a contamination event. This information is critical in risk assessment of food safety of fresh produce.

P3-52 Reduction of *Escherichia coli* O157:H7, *Salmonella* spp. and *Shigella* spp. in Parsley after Washing with Extracts of Edible Vegetables

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Introduction: *Escherichia coli* O157:H7, *Salmonella* spp and *Shigella* spp have been associated with foodborne diseases after consumption of contaminated leafy greens vegetables. Several plant extracts have proven effective in inhibiting the growth of pathogenic bacteria present in vegetables, and could be an alternative to reduce or eliminate the microbial load of pathogens.

Purpose: To reduce the level of *E. coli* O157:H7, *Salmonella* spp and *Shigella* spp in parsley after washing with extracts of edible vegetables.

Methods: Twenty aqueous and ethanolic extracts of edible plants were tested for antimicrobial activity against composite strains of *Salmonella*, *E. coli* O157:H7 or *Shigella* spp using the agar-well assay. The two most active extracts were selected and the minimum bactericidal concentration (MBC) determined. Mixtures of extracts were analyzed for synergistic activity against the strains using the *checkerboard method*. The mixture with synergism was used to wash 60-g parsley samples previously inoculated (by spot method) with 10^3 cells/ml. After 0, 1, 3, 5 and 7 days, viable bacterial counts were determined. 200 ppm chlorine, 200 ppm Citrol® and water were used as controls.

Results: Four extracts inhibited all the pathogenic bacteria tested. The extracts E1 and E2 (MBC of 1.5 mg/ml and 4.5 mg/ml, respectively) were selected for further experiments. The mixture (E1: 0.18 mg/ml and E2: 1.25 mg/ml) exhibited synergistic antimicrobial effect against a cocktail of each bacteria and was used for the washing procedures. A reduction of 2 log in *E. coli* O157:H7 and *Shigella* spp was observed at the first day and at the third day for *Salmonella*. These extracts were similar in efficacy to chlorine.

Significance: The extracts of two edible vegetables exhibited antimicrobial activity against *Salmonella* spp, *E. coli* O157:H7 and *Shigella* spp. and could be alternatives to reduce these pathogens in parsley.

P3-53 Inactivation of Microbes on Blueberries in Recycled Water Wash Systems

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Introduction: Washing fruit in water during postharvest processing is a common practice to remove harmless extraneous material. Chlorine and other sanitizers are used at various concentrations in water to inactivate spoilage and other microbes on fruit and in water. Typical systems consist of a bath or flume containing 100 s to >1000 l of water, which is often recycled.

Purpose: The purpose of this study was to examine the microbial load of wash water and fruit relative to water quality and sanitizer concentration in actual processing operations.

Methods: Water and fruit samples from six blueberry (BB) processors were collected at timed intervals and analyzed for microbes and various water quality parameters. *Escherichia coli* and spoilage microbes in water and on blueberries (BB) exposed to chlorine, chlorine dioxide, or peroxyacetic acid were enumerated using standard methods.

Results: Sanitizer concentrations fluctuated over time and in some cases decreased to 0-50% of the target residual. Turbidity values ranged between 0 to >200 NTU at the beginning and end of washing, respectively, and 70% of wash water samples had higher microbial counts compared to raw water. Microbial inactivation on BBs ranged from 29% to >99.9%, and 59% of washed BBs had lower microbial counts compared to raw BBs. In some cases, the microbial load of washed BBs increased by 1-3 orders of magnitude, in which levels of *E. coli* increased by 288%.

Significance: Water quality parameters change appreciably when wash water is recycled. Data suggests that maintaining a stable, target sanitizer residual concentration in wash water is problematic, due to oxidant demand in recycled systems. Though the microbial load is typically reduced on the fruit, microbial counts may be exacerbated on washed BBs.

P3-54 Kitchen Utensils as Tools to Remove or Transfer Bacterial Pathogens from Fresh-cut Produce

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Introduction: Utensils used in preparation of fresh-cut produce in the kitchen may serve as a vehicle for cross-contamination.

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Purpose: To advise consumers on food preparation practices that could affect the risk of contamination in the kitchen, studies were conducted that monitored the fate of bacterial pathogens on fresh-cut produce items that were subjected to brushing, peeling, grating, or cutting using common kitchen utensils.

Methods: GFP-labeled *Escherichia coli* O157:H7 and *Salmonella* spp. were spot-inoculated (50-150 µl of a 6-7 log CFU/ml inoculum) onto cantaloupe, honeydew melon, carrot, or celery prior to brushing, peeling, cutting, or grating. Bacterial enumeration or detection by enrichment culture was conducted on the utensils as well as the brushed or peeled produce items. The potential to contaminate non-contaminated produce items subsequently used with the contaminated utensil was also evaluated.

Results: Removal of bacterial pathogens by brushing of contaminated cantaloupes, honeydew melons, and carrots ranged from 0.1-0.6, 1.4-3.0, and 0.7-2.8 log CFU/100 g, respectively. Peeling carrots and celery led to pathogen losses ranging from 2.1-3.2 and 2.0-2.8 log CFU/100 g, respectively. A scouring pad used to brush produce items led to the highest incidence of contamination (57 positive of 89 items tested) whereas the nylon brush was the least likely to be contaminated (7 positive of 120 items tested). Transfer of bacterial pathogens to graters occurred 100% of the time but their transfer to knives ranged from 40% for tomatoes to 3% for cantaloupes. Contaminated knives and graters consistently transferred the pathogens to seven consecutive tomatoes or carrots being cut or grated whereas contaminated polyester-bristle brushes did not transfer the pathogens to carrots subsequently brushed.

Significance: In quantifying the cross-contamination risk associated with various steps in the food preparation process, the data collected in this study will aid risk management efforts in both home and food service kitchens.

P3-55 Low-energy X-ray Irradiation for Inactivating *Escherichia coli* O157:H7 in Date Paste

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Introduction: Dates (*Phoenix dactylifera* L.), like any other agricultural produce, are subject to potential microbial contamination in the field and during handling; however, there typically is no treatment applied to the dates that would be lethal to bacterial pathogens. Therefore, post-harvest contamination, such as by *Salmonella* or Shiga toxin-producing *Escherichia coli*, could persist until consumption.

Purpose: The objective of this study was to quantify *Escherichia coli* O157:H7 inactivation in date paste subjected to low-energy X-ray treatment.

Methods: Vacuum packaged whole dried dates were obtained from the Date Palm Research Center (Saudi Arabia) and were stored at 4 °C until tested. For the pathogen challenge test, a six-serovar cocktail of *Escherichia coli* O157:H7 was prepared, centrifuged, and re-suspended in sterile peptone water. The inoculum was mixed with 200 g of dates to be ground. The inoculated and ground sample (~3 g) was placed in a plastic bag and rolled to a 1 mm thickness to maximize X-ray dose uniformity. Five dose levels of X-ray (70 kV, 57 mA) were applied to achieve 1 to 5 log reductions in triplicate. After irradiation, samples were homogenized and plated on Petrifilms™ *E. coli*/Coliform Count Plates (3M, St. Paul, MN), with colonies enumerated after incubation at 35 ± 2 °C for 48 h.

Results: Initial concentration of *E. coli* O157:H7 was 7.42 log CFU/g in the date paste. The reciprocal of the slope of the regression line was calculated as the D_{10}-value (0.37 ± 0.04 kGy). Based on this measurement, a 5-log reduction can be attained with 1.85 kGy. The X-ray treatment had minimal effect on the visual color of the date paste.

Significance: Low-energy X-ray was able to inactivate *E. coli* O157:H7 in date paste with a reasonable dose, suitable for potential commercial operations. Therefore, low-energy X-ray is considered as a viable method for ensuring microbiological safety of this low-moisture product.

P3-56 Pathogen Prevalence and Indicator Organism Levels in Three Open Surface Water Systems in Washington

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Introduction: Understanding pathogen risk associated with irrigation water is an important aspect of produce food safety.

Purpose: To examine open surface waters as a potential source of microbial contamination for produce farming systems, three water systems in Washington were monitored for pathogen presence and indicator organism levels over two years.

Methods: Sampling sites were selected based on stakeholder input to collect monitoring data that would be beneficial to local producers. Site A (8 sampling sites and 18 sampling dates), Site B (14 sampling sites and 13 sampling dates) and Site C (16 sampling sites and 12 sampling dates) represented three different geographic regions in Washington. Samples were quantified for fecal coliforms and generic *Escherichia coli* using a five-tube most probable number technique, and analyzed for pathogen presence (*E. coli* O157, *Salmonella*). Washington Department of Ecology (WA-DOE) utilizes a water quality standard for secondary contact recreation water of < 2.3 log colonies/100 ml fecal coliforms and the Leafy Greens Marketing Agreement (LGMA) recommends <2.37 log MPN/100 ml generic *E. coli* for foliar irrigation application.

Results: Pathogen prevalence (*E. coli* O157 and *Salmonella*) ranged from 5.2% (5 pathogen positives) at Site A to 6.9% (14 pathogen positives) at Site C. At Site A, 5 pathogen positives were observed when both the WA-DOE fecal coliform standard and LGMA *E. coli* standard were met, and only 1 pathogen positive was observed when both standards were exceeded. Alternatively, both Sites B and C had 5 pathogen positives observed when both WA-DOE and LGMA standards were exceeded. Site B had 6 pathogen positives when only the WA-DOE fecal coliform standard was exceeded and Site C had 9 pathogen positives when only the WA-DOE fecal coliform standard was exceeded.

Significance: Meeting indicator organism standards may not accurately reflect pathogen risk. Emphasis on good agricultural practices and risk assessment for irrigation water management programs is warranted.

P3-57 Efficacy of Aerosolized Chlorine Dioxide in Reducing *Salmonella* Typhimurium on Food Surfaces

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Introduction: Aqueous chlorine dioxide (CD) has a limited effect on kill pathogens on food surfaces. Also, gaseous CD needs sophisticated equipment and has limited legal application. To overcome the disadvantages of aqueous or gaseous CD, an aerosolized CD prepared with a nebulizer would have a better diffusivity and permeability on food surfaces so that it would be effective method for controlling the pathogen.

Purpose: The purpose of this investigation was to study kinetics of inoculated *Salmonella* Typhimurium on tangerine and green pepper by aerosolized CD treatment and to determine the effect of aerosolized CD on the quality and residual concentrations on the produce.

Methods: The tangerine and green pepper inoculated with *Salmonella* Typhimurium were treated to various concentrations of CD which was aerosolized by use of jet and ultrasonic nebulizer for different treatment times. The antimicrobial activity of the aerosolized CD prepared by jet nebulizer and ultrasonic nebulizer were compared.

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Results: In the CD generation efficiency, the phosphoric acid as an acidulant to react with sodium chlorite produced relatively higher concentration of CD than the other acids and the purity of CD was 96~98%. Bacterial numbers reduced as increase of aqueous chlorine dioxide concentration and treatment time. In the treatment of aerosolized CD prepared by jet nebulizer, the bacterial reductions on tangerine and green pepper were 2.98, 3.12 log for 60 min at 300 ppm of CD, 3.81, 4.21 log for 60 min at 400 ppm and D-values were 16.47 and 14.84 min, respectively. However, aerosol CD by ultrasonic nebulizer treatment for 60 min at 300 and 400 ppm on tangerine and green pepper surface showed undetectable levels of *S.* Typhimurium and D-values were 5.04 and 4.92 min, respectively. Residual concentrations of tangerine and green pepper were less than 1.5 ppm. Aerosolized CD treatment caused negligible changes in Hunter color L, a, and b values and did not affect the color of tangerine and green pepper during storage.

Significance: These results indicate that aerosolized CD treatment can be useful in improving the microbial safety and quality of fresh produce.

P3-58 Survival of *Escherichia coli* and *Salmonella* spp. in Soil Treated by Biosolarization Method

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Developing Scientist Competitor

Introduction: Traditionally, pest control and fertilization of the fields are carried out through the application of pesticides and inorganic fertilizers. However, in recent years the trend worldwide focus in reducing the use of these compounds. Biosolarization process is a non-chemical disinfection technique used to control soil pests and plant pathogens. The method is based in the approach of combining microbial degradation activity of complex substances that come from organic waste (plant residues, animal manure) and solarization that involves covering soil with a clear plastic film to trap solar radiation and accumulate heat. It is necessary to generate information regarding the effectiveness of biosolarization in the inactivation of foodborne pathogens.

Purpose: The purpose of the study was to study the efficacy of biosolarization in the inactivation of *Escherichia coli* and *Salmonella* inoculated in soil.

Methods: Soil was mixed with cattle, goat and sheep manure (120 ton/ha) and hydrated to field capacity. One group of samples was inoculated (ca. 7 log CFU/g) with *Salmonella* spp. resistant to rifampicin; another group of samples was inoculated with *E. coli* (ca. 6 log CFU/g) containing a gene to express the green fluorescent protein (GFP). Inoculated samples were stored at 45 and 25 °C for up to 6 weeks.

Results: At 45 °C (biosolarization) *E. coli* and *Salmonella* were not detected after four days of storage. At 25 °C both microorganisms survived for up to 6 weeks; at the end of the storage the decrease in the population of *E. coli* and*Salmonella* was 3 and 5 log CFU/g, respectively.

Significance: Biosolarization represents a viable alternative to inactivate foodborne pathogens such as *Salmonella* in the short term. Additionally, the treatment reduces the incidence of phytopathogens and increase the nutrient content in soil.

P3-59 Rapid Detection of Yeast and Mold in Filterable Beverage Using PCR Assay

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Introduction: Fungal spoilage of food accounts for 5% - 10% of all losses in global food production. This spoilage is not only a quality issue, but also food safety concern since many common mold species are dangerously toxigenic. Due to this concern, it is imperative that manufacturers closely monitor their processes to prevent such contamination from occurring. While traditional colony count methods for yeasts and molds require 5-7 days to achieve a result, the Yeast and Mold PCR assay provides same day results for food samples containing > 500 CFU/g (direct method), or in just 2 days for food samples with 50-500 CFU/g (enrichment method). This study was to validate Yeast and Mold PCR Assay in filterable beverage for the detection of yeast and mold at very low level.

Purpose: The objectives of this study were to validate the PCR assay for detecting low levels (1 CFU/5ml) of yeast and mold in filterable beverage and to compare the efficiency of sample processing through both filtration and centrifugation to collect yeast and mold cells.

Methods: Two master samples of soda were spiked with *Saccharomyces cerevisiae* and *Aspergillus niger* at a level targeting 1 CFU/5ml. Samples were either filtered using in-line filter units or subjected to centrifugation, then all samples were incubated at 25 °C for 44 hrs. Samples were prepared for yeast and mold detection and a full process was run on the BAX® System instrument according to the procedures described in the BAX® System User Guide.

Results: All spiked samples regardless of the sample processing method used, returned positive results with the PCR assay for the target organism, and all non-spiked samples returned negative results. Actual spiking levels for inoculated samples was determined to be 0.6 CFU/ml for *S. cerevisiae* and 0.14 CFU/ml for *A. niger* after the 44-hour incubation.

Significance: This study demonstrates that the PCR assay can detect yeast and mold in soda within two days at only 1-3 CFU/5 ml. Sample processing using filtration is equivalent to centrifugation to collect cells from filterable beverages. This study also demonstrates that the PCR assay can detect yeast and mold in filterable beverages, provided that at least 1 CFU is retained by the filter.

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P3-60 Antimicrobial Efficacy of Clarity® (Peracetic Acid) with a Booster (Peradigm®) against *Chaetomium globosum*

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Introduction: Thermotolerant spoilage fungus *Chaetomium* is of particular concern in the beverage filling industry. This persistent organism has been suggested in Japan as an indicator organism for aseptic filling of PET bottles. Antimicrobial agents, such as peracetic acid (PAA) sold as Clarity® (FMC Corporation) can be used to address this concern. The efficacy of PAA can be enhanced by use of the additive Peradigm® (FMC Corporation).

Purpose: Tests were conducted to assess the antimicrobial efficacy of PAA between 2000 and 4000 ppm with and without Peradigm® to reduce *Chaetomium globosum* ATCC 6205 inoculated onto carriers.

Methods: PAA concentration, addition of booster, contact time, and temperature, were evaluated in tests performed using carriers. Stainless steel strips inoculated with spores of *C. globosum* were dried overnight and tested against 2000 to 4000 ppm PAA with and without Peradigm®, with 10 and 30 s contact times at 55 and 60 °C. The strips were neutralized in Letheen broth with sodium thiosulfate, were sonicated and vortexed. The resulting solutions were diluted and plated on Petrifilm™ YM plates, incubated 5 days at 30 °C and then counted.

Results: Greater reduction was achieved using Peradigm®, with complete kill (5.71 log reduction) achieved using a 1:50 ratio of Peradigm® with 4000 ppm PAA at a contact time of 30 seconds at 55°C, compared with a 1.57 log reduction without using Peradigm®. At 60 °C, all samples tested

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at 2000, 3000, and 4000 ppm PAA with 1:50 Peradigm® resulted in complete kill at 30 seconds.

Significance: *C. globosum* (ATCC 6205) demonstrated persistence despite the presence of heat and biocide, comparable to or greater than some bacterial endospores previously tested. Clarity® at concentrations between 2000 and 4000 ppm PAA was able to achieve complete reduction of this organism using the booster product Peradigm®. Use of Peradigm® was critical in achieving complete reduction of the organism at a lower test temperature of 55 °C, preferable when using PET bottles, to avoid softening or deformation.

P3-61 Why Do People Prefer Bottled Waters?

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Introduction: Polyethylene Terephthalate (PET) bottles were introduced for using in carbonated waters in 1973. Bottled waters are described as "waters that were packaged in bottles or similar materials for consumption by people." Bottled waters are mainly prefered because of their easy usage and transport.

Purpose: The aim of this study is to determine the bottled water consumption attitudes of students in a boarding school.

Methods: This descriptive study was performed on juniors in a boarding school, and a total of 85 participants were included. A questionaire developed by the authors was applied to participants, and results were analyzed using SPSS 15.0.

Results: Almost all of the participants (98%, n=84) were using bottled waters with a volume of 1,500 ml during daily activities, and 88% of them declared that they had been using bottled waters for three years. According to their statements, they prefer bottled water. 37% believe that bottled waters are healthier than tap water, and 69% stated that bottled water is easily available. A big portion of participants (74%) did not drink tap water because they believe that tap water is dirty. Most of them did not use a glass while drinking bottled water and drank directly from the bottle.

Significance: This study showed that bottled waters were prefered by students mainly because of their belief that bottled waters were healthier, and because of the practicality of usage. Participants did not prefer tap water, because they did not believe that it is hygienic.

P3-62 Efficient Reduction of *Escherichia coli* from Apple Cider by Combining Microfiltration with Ultraviolet Treatment

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Developing Scientist Competitor

Introduction: Consumption of raw apple juice or cider contaminated with *E.coli* O157:H7 has resulted in several outbreaks in recent years. Thermal pasteurization can achieve a 5-log reduction of this pathogen, but can negatively affect the nutritional and organoleptic properties of juice. Ultraviolet (UV) treatment is a FDA recognized alternative to thermal processing of apple juice and cider, but the suspended solids in the treated product can limit its efficiency. Microfiltration (MF) can be used to physically remove suspended solids and microorganisms from apple cider, thus enhancing the effectiveness of UV, and allowing a lower UV dose to be used.

Purpose: In this study, the efficiency of a combined UV & MF treatment in the reduction of *E.coli* from apple cider was investigated.

Methods: Apple cider with pH 3.7 and 12.7 °Brix was used as the raw material. The cider was inoculated with both pathogenic and non-pathogenic strains of *E. coli*, at more than 10^7 CFU/ml, and then subjected to the individual or combined treatments. MF was performed with a 0.8 µm ceramic membrane, at 6 °C and 105 kPa transmembrane pressure. The UV treatments were conducted using a CiderSure 3500 reactor, at a low UV dose of 5 mJ/cm^2. Microbial counts and chemical composition before and after processing were determined.

Results: MF resulted in more than 5-log reduction of *E. coli* in the permeate, as did UV. The penetration depth of UV was improved by the MF step, and as a result the combined MF & UV treatment led to a cumulative reduction of *E. coli* of over 7 log. The data also suggests that the UV dose could be further reduced.

Significance: The developed non-thermal hurdle treatment has the potential to significantly reduce pathogens in apple cider, as well as spores, yeasts, molds and protozoa, and thus help juice processors improve the safety and quality of apple juice and cider, as well as other fruit juices.

P3-63 *Withdrawn*

P3-64 Are Restaurant Employees Aware of Food Allergens?

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Developing Scientist Competitor

Introduction: In order to improve food safety and confidence in restaurants' allergen awareness, training and knowledge are paramount. Poor training or lack of knowledge of food allergies poses significant risks to patrons. Advances in modern medicine have solidified the links between certain foods and sensitivities and diseases, as well as increased ability to test for certain allergens. As a result, consumers are increasingly aware of their susceptibility to react to specific foods and an increasing number of patrons are requesting special handling of food for allergies or intolerances.

Purpose: The objective of this study was to evaluate food handlers' and managers' current knowledge of food allergens.

Methods: A convenience sample of 264 food service employees and managers who were also students enrolled in a food service course at a major U.S. University was selected. Participants were provided a modified version of the Food Allergen Knowledge and Practices survey. Participants made their ratings on 15 items intended to measure the participant's overall knowledge regarding food allergies and practices. Sample questions included: Can high temperature destroy food allergens? If you remove allergenic food items from a dish, will it prevent the customer from having an allergic reaction? Is direct ingestion the only way an allergen can be introduced to a person?

Results: Results indicated an overall deficit of knowledge of food handlers and managers regarding food allergens; more specifically, general knowledge identifying the 8 major food allergens, proper handling and cross-contact risks of allergenic foods, general knowledge of anaphylaxis and that it can be fatal, and crisis management for harmful reactions.

Significance: This study raises question as to the adequacy of food safety training regarding allergens. Many food allergy mishandlings are preventable; however, employee training needs significant improvement. The results of this study could be used to prepare more effective allergy training material.

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P3-65 Food Safety Priorities for Retail Deli Managers

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Developing Scientist Competitor

Introduction: In recent years, *Listeria monocytogenes* has emerged as one of the largest food safety concerns due to the increase in consumption of ready-to-eat foods vulnerable to contamination post cooking (Garrido, Vitas, & Garcia-Jalon, 2009). With the seriousness of foodborne illness risk in the deli industry, more emphasis has been placed on food safety training for deli employees.

Purpose: The objective of this research was to identify which food safety priorities deli managers consider relevant and important to include in specific food safety training programs developed for deli workers. This information may provide the person in charge (PIC) of food safety for delis and food safety instructors in deli operations guidelines for implementing these practices.

Methods: An evaluation matrix consisting of food safety objectives was built based on the United States Food and Drug Administration (FDA) Food Code and four food safety training modules currently available online: the National Restaurant Association's Educational Foundation's ServSafe®, FMI's Super Safe Mark®, Training Achievement Programs®, and Alchemy Systems. In order to solicit participation from a variety of subject experts and to generate accurate and authentic ideas from the experts in the field, a three round modified Delphi technique using Qualtrics software was conducted by 15 experts currently working or managing in retail delis.

Results: The results found that employee safe food handling practices (glove use, avoiding cross-contamination) preventing foodborne illness such as time/temperature control, cross-contamination, cleaning and sanitizing are the most salient objectives as perceived by experts for food safety training in deli industry.

Significance: The findings indicate that trainers and managers need to emphasize specifically employees' safe food handling practices, time/temperature control, and cross-contamination when purchasing existing food safety training materials or developing new food safety training programs internally.

P3-66 Dietary Intake of Preservatives, Antioxidants by Korean Population

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Introduction: To investigate the intake of preservatives and antioxidants, 600 items of preservatives and 400 items of antioxidants were purchased as monitoring sample, and the amount of preservatives and antioxidants in 14 and 8 items, respectively, was analyzed. The intake level and its safety were assessed through comparison with the ADI suggested in JECFA. As a result of monitoring preservatives and antioxidants, preservatives were detected in 304 out of 610 samples of 37 items and as a result of analyzing 418 samples of 21 antioxidant items, a large amount of erythorbic acid and EDTA was detected. As a result of intake assessment according to the Korean population's sex, age, and consumer group, the intake of preservatives and antioxidants was found to be at a safe level, *i.e.*, less than 30% of the ADI suggested by JECFA.

Purpose: The main purpose of this study is to suggest risk management and risk communication strategy based on the transparent and fair analysis of food safety through dietary exposure assessment by Korean population under application of assessment model developed in 2009.

Methods: To investigate the intake of preservatives and antioxidants, 600 items of preservatives and 400 items of antioxidants were purchased as monitoring sample, and the amount of preservatives and antioxidants in 14 and 8 items, respectively, was analyzed. Additionally, the intake of preservatives and antioxidants was calculated by applying to the result of monitoring the result of the National Health and Nutrition Survey 2009 (8,921 persons) and the Children's Food Intake Survey for Two Days Each Season in 2007-2008 (6,625 persons); the intake level and its safety were assessed through comparison with the ADI suggested in JECFA.

Results: As a result of monitoring preservatives and antioxidants, preservatives were detected in 304 out of 610 samples of 37 items and used for a food type containing the most sorbic acid. Sorbic acid was detected in processed meat, processed fish, salted food, etc., and benzoic acid was detected in beverages. As a result of analyzing 418 samples of 21 antioxidant items, a large amount of erythorbic acid and EDTA was detected. Both preservatives and antioxidants were appropriate for the utilization standard. As a result of intake assessment according to the Korean population's sex, age, and consumer group, the intake of preservatives and antioxidants was found to be at a safe level, *i.e.*, less than 30% of the ADI suggested by JECFA.

Significance: The intake of preservatives and antioxidants was not more than ADI in both the group that eats processed food containing preservatives and antioxidants on the average and the group (95th from consumers) within the group that eats processed food containing preservatives and antioxidants to the extreme; in the 95th consumer, however, benzoic acid was at a high level, *i.e.*, 35.7% of ADI. Therefore, the intake of preservatives and antioxidants was usually at a safe level.

P3-67 Economically Motivated Adulteration: Detection of Anomalies in the Supply Chain through Monitoring of Import Data

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Introduction: Economically-motivated adulteration (EMA) is the adulteration of food for financial advantage. Adulteration of wheat gluten with melamine in 2006 and 2007 resulted in animal deaths and impacted the U.S. food production system. EMA events reveal vulnerabilities in our food system that could be exploited for intentional harm.

Purpose: The purpose of this study was to retrospectively apply early event detection (EED) techniques based on the field of statistical process control to line-entry import data maintained by U.S. Customs and Border Protection (CBP) to determine if these methods could have detected a signal that would have indicated an anomaly in the supply chain for wheat gluten. An EED signal could have alerted regulatory authorities prior to illness reports.

Methods: Line entry data of wheat gluten for animal feed imported from China from 2003 – 2007 were obtained from CBP. The CUSUM control chart methodology was applied to daily quantities of wheat gluten imports after adjusting for systematic trends in the data. A threshold was applied to optimize the balance between sensitivity of signal detection and minimization of false signals.

Results: The analysis covered 543 shipments imported on 374 of 1,631 total days of data. The average quantity per shipment was 69,000 kg (std. dev., 40,000 kg). A threshold was chosen to achieve a minimum time between false signals of approximately one month. Multiple signals were detected in 2006: The first was detected seven months prior to the first known animal illnesses and the last was detected two months prior to the first known animal illnesses.

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Significance: Monitoring of line-entry import data has the potential to alert us to anomalies in the supply chains for food ingredients. This approach can be used by the private sector and regulatory authorities to monitor imports in real time to target limited inspection and testing resources to the highest risk food ingredients. Moving forward, integrated EMA scoring models that incorporate trade data, QA methods, supply chain structure, and pricing can predict EMA potential.

P3-68 The Association between Menu Labeling of Common Allergens and Food Safety Knowledge and Attitudes: A Study of Independently-operated Restaurants

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Introduction: Food allergies or adverse immune responses to certain foods affect about 12 million Americans. While many locales require or encourage the use of warnings for foods at high risk for foodborne pathogens, labeling of common food allergens on menus is typically an optional, voluntary practice for U.S. restaurant owners.

Purpose: The purpose of the study was to examine whether menu labeling of allergens is associated with restaurants whose owners/operators have higher levels of food safety knowledge and whether menu labeling is associated with differing perceptions of restaurant and customer responsibility for food safety.

Methods: A survey was developed, validated and mailed to a national sample of 3,000 independently owned and operated U.S. restaurants.

Results: Only 43 (19.9%) of 220 respondents indicated that their restaurant labeled food allergens on menus. A majority of all respondents (88.1%) felt that it was the customer's responsibility to order food that was allergen-free. Overall, 41.9% of respondents agreed that wait staff would know the ingredients of all menu items served at their restaurant while 68.5% agreed that kitchen staff would know the ingredients. However, those who labeled food allergens on menus were significantly more likely ($P < 0.01$) to) to believe that restaurants were also responsible for providing allergen-free foods and that customers should be warned about foods containing common food allergens. They also were significantly more likely ($P < 0.10$) to identify the importance of handwashing for prevention of foodborne illness and food allergen reactions.

Significance: Menu labeling of allergens may indicate a general awareness of food allergen issues on the part of restaurant owners/operators. Since food allergies are a serious matter for many consumers, it is important to increase awareness and education of restaurant employees regarding menu ingredients and food handling practices that ensure allergen-free foods.

P3-69 Food Safety Culture in Healthcare Foodservice Operations

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Introduction: Hospitals and other healthcare foodservice departments serve significant numbers of people who are most susceptible to foodborne illnesses. It is extremely important to provide adequate food safety training for the workers to ensure the safety of food served in healthcare institutions. Even though it has been shown that food safety training can increase knowledge, this knowledge does not necessarily change food handling practices. Food safety culture has been proposed as a better way to change the behaviors and to create more effective food safety systems. By changing employee behavior, foodservice workers will ultimately be providing safer food and may reduce the risk of foodborne disease to patients, staff and guests.

Purpose: The objective of this study was to determine what factors are needed to create a positive food safety culture in healthcare foodservice departments from the management perspective.

Methods: Managers and supervisors from selected healthcare foodservice facilities were given an online version of the Food Safety Climate Tool modified for healthcare foodservice departments. Participants rated 38 items using a 5-pointLikert-type scale, 1 (strongly disagree) to 5 (strongly agree). Items were related to five food safety areas: (1) management commitment to food safety (leadership and resource allocation); (2) work unit commitment to food safety (supervisor, co-worker and personal commitment); (3) food safety training; (4) infrastructure for food safety (food safety management system and production practices); and (5) worker food safety behavior.

Results: The most important factors for developing a food safety culture in healthcare were management commitment and foodservice worker behavior. It was also found that management must allocate resources through training, provide adequate tools (thermometers, food labels), and exhibit signs of active management control within the foodservice operation.

Significance: Utilization of these results will help develop tools for foodservice managers to create improved food safety culture and help improve compliance of foodservice workers to follow proper food handling practices.

P3-70 Potential Use of DNA Barcodes in Regulatory Science: Identification of FDA's "The Dirty 22"

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Introduction: The Food, Drug, and Cosmetic Act (FD&C Act) prohibits the distribution of food that is adulterated, and the regulatory mission of the U.S. Food and Drug Administration (FDA) is to enforce this Act. FDA field labs have identified the 22 most common pests that contribute to the spread of foodborne disease. The current method of detecting filth and extraneous material (tail, leg, carcass, etc.) is done visually using microscopy. Because microscopy can be time consuming and may yield inaccurate/non-specific identifications due to the expertise needed, an alternate molecular method is needed.

Purpose: To sequence DNA from the 5' region of cytochrome oxidase I (COI) (DNA Barcode) of 22 of the most common pests ("The Dirty 22") contributing to the spread of foodborne pathogens. The targeted pests are: German cockroach, Brownbanded cockroach, Oriental cockroach, American cockroach, Pharaoh ant, Thief ant, House fly, Stable fly, Little house fly, Latrine fly, Cosmopolitan blue bottle fly, Holarctic blue bottle fly, Oriental latrine fly, Secondary screwworm, Blue bottle fly, Green bottle fly, Black blow fly, Redtailed flesh fly, House mouse, Polynesian rat, Norway rat and Roof rat.

Methods: Genomic DNA of the targeted 22 species was extracted and the barcode region of the COI gene was amplified and sequenced.

Results: DNA barcodes were generated for 21 of the 22 species. Fannia scalaris (Latrine fly) did not yield suitable barcodes due to the condition of the archival specimen used, as a result of the fixative agent used to store the specimen, which prohibited DNA extraction.

Significance: Our study demonstrates that DNA barcoding can be a powerful tool for species identification and has broad regulatory applications. To date, this is the first molecular assay to attempt to identify the FDA targeted 22 species and has the potential to aid the U.S. Food and Drug Administration's initiative to provide protection and safety to the U.S. food supply.

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P3-71 On the Implementation of a Food Safety System in a Small Dairy Processing Factory in State of Sao Paulo, Brazil

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Introduction: The good manufacturing practices (GMPs), the sanitation standard operating procedures (SSOPs) and the hazard analysis and critical control points system (HACCP) comprehend the basis of a food safety system. In Brazil, implementation of the HACCP system in small dairies is of fundamental importance since this segment supplies dairy products to a large proportion of the population.

Purpose: The purpose of this study was to describe the implementation and costs of a food safety system in a small dairy factory.

Methods: The study was carried out in a dairy factory located in the central region of the State of São Paulo, Brazil. The steps involved in the implementation of the food safety system included a diagnosis of the prerequisites, implementation of the GMPs, SSOPs, training of the food handlers and HACCP. The total cost of implementing the systems was assessed using the method of cost appropriation (accounts for direct and indirect expenses). The total count of yeasts and molds was used to assess the impact of the implementation of food safety system in the dairy factory.

Results: In the initial diagnosis conformity with 70.7% (n=106) of the items analyzed regarding GMP was observed. A total of 12 critical control points (CCPs) were identified. After implementation of the food safety system, a significant reduction in the yeast and mold count was observed ($P < 0.05$). The total cost of implementing the food safety system was US$ 61,812.00, signifying an impact of US $3.81/t of yogurt packed (0.5% of the production costs). The resources used for investment signified an additional US$ 12.92/t (1.5% of the cost per kg of yogurt packed).

Significance: The implementation of the food safety system assessed in this study presented an adequate cost-benefit relationship, which would be enhanced with the improvement and sedimentation of the culture of food safety in the company.

P3-72 Photodegradation of Aflatoxin B1 in Food

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Introduction: Aflatoxins, a group of highly toxic, mutagenic and carcinogenic compounds, are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and Aflatoxin B_1 (AFB_1) is classified as a Group1 carcinogen by the International Agency for Research on Cancer (IARC). UV irradiation has been known for many years as an effective physical method for the destruction of aflatoxins due to their photosensitivity. The exact nature of the photodegradation products of aflatoxins remain unknown and whether or not they are harmless substances has become a major cause of apprehension toward this advanced photochemical detoxification method. Photodegradation of Aflatoxin B_1 (AFB_1) in peanut oil and water media were studied and the photodegradation pathways & the toxicity of the photodegradation products in these two medias were proposed and identified, respectively.

Purpose: The objectives of this study were to verify the effectiveness and the safety of the detoxification of AFB_1 in the food media, peanut oil and water, by UV irradiation.

Methods: The photodegradation behaviors and the photodegradation pathway were studied by UPLC-Q-TOF/MS technology. The Ames test, employing *Salmonella* Typhimurium tester strains TA98 and TA100, was employed to evaluate the residual toxicity of the AFB_1 sub-products in the two media. Cytotoxicity, apoptosis, and p53 expression were assessed by MTT, Flow Cytometric Analysis , and ELISA Assay kit in Hep G2 cells lines.

Results: The photodegradation behaviors of the AFB_1 in these two medias were proposed in this study. Some photodegradation products were identified by UPLC-Q-TOF/MS and the degradation pathways were proposed in these two medias. The results of the Ames test and *in vitro* toxicity test indicated that the mutagenic activity and the cytotoxicity of UV treated samples in peanut oil have been destroyed absolutely, while the cytotoxicity of UV treated samples in water have been weakened but not completely abolished, which are in agreement with the elucidation of the structures of the photodegradation products of AFB1 in these two medias.

Significance: In the present study, the mutagenesis and *in vitro* toxicity of AFB_1 and its photodegradation products towards HepG2 cells was evaluated. This study, which provides an initial risk assessment of the UV irradiation detoxification method at a genomic and cellular level, is very useful for future studies on the mechanism of AFB_1 under UV irradiation.

P3-73 Survival of *Salmonella* during Baking of Peanut Butter Cookies

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Introduction: Peanuts and peanut-based products have been the source of recent *Salmonella* outbreaks worldwide. Since peanut butter is widely used as an ingredient in baked goods, the risk of *Salmonella* remaining in these products after baking needs to be assessed.

Purpose: This research examines the potential hazard of *Salmonella* in peanut butter cookies when it is introduced via the peanut-derived ingredient. The objective was to determine the survival of *Salmonella* during the baking process.

Methods: Commercial, creamy-style peanut butter was artificially inoculated with a 5-strain *Salmonella* cocktail at a high (10^8 CFU/g) and a low (10^5 CFU/g) target concentration. Peanut butter cookies were prepared using a standard recipe and the inoculated peanut butter. Initial *Salmonella* concentrations in the dough made with the high and low inoculated peanut butter were 6.5 and 2.5 log CFU/g, respectively. Dough was baked at 350 °F for 15 minutes. After baking, the survival of *Salmonella* was determined either by plating or enrichment. Temperature profiles of the cookies were monitored during baking. Cookie moisture content, water activity and pH were measured before and after baking.

Results: *Salmonella* survived in 13 of the 24 samples prepared with the high inocula peanut butter. In positive samples, *Salmonella* populations decreased an average of 5.0 log CFU/g. No *Salmonella* was detected in the 24 baked samples made with the low *Salmonella* contaminated peanut butter, resulting in a decrease greater than 3.9 log CFU/g.

Significance: Results of this study indicate that the risk of *Salmonella* in peanut butter cookies prepared with contaminated peanut butter is greatly reduced during baking.

P3-74 Evaluation of Domestic Distribution and Safety of Fresh Ginseng (Panax Ginseng C.A. Meyer) in Korea

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Introduction: Fresh ginseng has always been well-known and widely used in traditional Oriental medicine. Its popularity with the general public is also on the rise. Potential demand for ginseng and ginseng products as a food ingredient is increasing, which make safety concerns over

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the often soiled, fresh roots all the more serious.

Purpose: This study aims to address consumer concerns for hygiene by carrying out a series of food safety tests on fresh ginseng in circulation.

Methods: This study monitored the microorganisms, pesticide residue, and heavy metals of fresh ginseng products in its places of production, traditional markets, wholesale markets and department stores in Korea. The ginseng was analyzed separately by part: the rhizome head, the main root and the lateral root+hair root.

Results: The fresh ginseng at all markets was uncleaned with surfaces or shapes in good condition. In general, ginseng sold at the production sites or traditional markets obtained better evaluation marks than that at the other locations. The microorganism test results showed that both the rhizome head (4.47 log CFU/g) and lateral roots+hair root (3.68 log CFU/g), as compared to the main root (2.34 log CFU/g), were more contaminated with coliform group. According to a pesticide residue analysis, the rhizome head is found to have the highest number of pollution cases 29 cases, followed by the main root of 7 cases, and lateral root+hair root of 8 cases. Meanwhile, Cu and Cr were detected from all production sites and Ni from some sites. The rhizome head was found to have the highest level of Cu with 5.4-13.8 mg/kg, followed by the main root with 2.4-3.7 mg/kg, and the lateral root+hair root with 4.4-6.6 mg/kg. The rhizome head and lateral root+hair root in the GP region are found to be exposed to Ni with 0.8 and 1.2 mg/kg, respectively.

Significance: The results of this study can be utilized as basic data for developing a ginseng industry that is competitive and highly value added.

P3-75 Causes and Consequences of Restaurant Closures for Food Safety Violations

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Introduction: With half of the food dollar being spent on food at retail foodservice establishments, it is critical that these establishments provide safe food. The health inspection process is one way that public health authorities can ensure this outcome. Health inspectors use objective metrics to judge whether safety and sanitation standards have been met. Ultimately, if an establishment has violations that seriously compromise the safety of the food offered for sale, that establishment may be closed. The economic consequences of restaurant closures are varied and can affect the viability of the business.

Purpose: The objective of this pilot study was to determine what factors led to the closure of restaurants by the health department and what the economic impacts to the establishments are.

Methods: Inspection records from counties representing different geographic regions of the country as well as rural and urban areas were examined to determine the reasons for restaurant closures. A sampling of media reports regarding these closures were also examined as a means of discerning potential economic impacts from these closures.

Results: The most prevalent reasons for restaurant closures were: operating without a valid health permit, no hot or potable water in the facility, vermin infestation and unsanitary equipment/utensils/food contact surfaces. Economics consequences associated with these closures include lost revenues during the period of closure, compliance costs and lost revenues due to reputation losses.

Significance: Determining the reasons for restaurant closures can provide health departments and food safety trainers needed information so they can concentrate on more specific factors and help to decrease the number of closures each year. Furthermore, the potential imposition of large costs on violators of food safety standards can create a powerful incentive for businesses to follow best practices, thereby leading to safer foods.

P3-76 What are Restaurant Managers' Priorities for Creating a Food Safety Culture?

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Developing Scientist Competitor

Introduction: By changing employee behavior to better handle food safety issues, restaurants will ultimately be providing safer food and may reduce the risk of foodborne disease to consumers. One approach to changing employee behavior is the development of a food safety culture. In our previous study, restaurant employees reported the importance of management taking safety seriously, showing leadership by keeping employees focused on food safety and modeling appropriate food safety behaviors.

Purpose: The objective of this study was to identify restaurant managers' priorities for developing food safety training and creating a food safety culture.

Methods: In this pilot study, a convenience sample of 12 foodservice managers was selected. Managers were given a modified Food Safety Culture Survey Tool with a total of 43 statements concerning workers commitment to food safety, management commitment to food safety, worker food safety behavior, food safety training, and infrastructure for food safety. For each topic area, managers were asked to rank a list of 6 statements with 1 being the highest priority for managers.

Results: Restaurant managers reported that the most important priorities for developing a food safety culture were: 1) employees following all the food safety rules even if no one is looking, 2) management being committed to serving safe food, 3) everyone in the establishment completely supporting the food safety program, 4) employees receiving proper training to follow the food safety rules, 5) and management visibly showing support for food safety ("walks the talk").

Significance: These results are consistent with our previous study and suggest that restaurant mangers realize that their commitment to food safety influences not only employee behavior but also employee attitudes toward creating a food safety culture.

P3-77 Purification and Partial Characterization of a Bacteriocin Produced by *Leuconostoc mesenteroides* A11

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Introduction: Bacteriocins produced by lactic acid bacteria are antimicrobial peptides, ribosomally synthesized, with potential application in the control of spoilage and pathogenic bacteria in foods, especially *Listeria monocytogenes*. These peptides have low toxicity compared to preservatives currently in use, due to their degradation by gastrointestinal enzymes. However, many bacteriocins have not been fully characterized and are not allowed yet for biopreservation.

Purpose: To purify and partially characterize the bacteriocin produced by *L. mesenteroides* A11 isolated from chicken breast.

Methods: Previous studies showed *L. mesenteroides* A11 is bacteriocinogenic and inhibits *L. monocytogenes*. In this study, the partially purified bacteriocin was obtained from the neutralized filter-sterilized culture supernatant of *L. mesenteroides* A11 grown in BHI broth at 25 °C for 24 h.

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For that, purification with Amberlite® XAD-16 resin, followed by solid phase extraction – SPE were employed. Fractions were obtained with a 2-propanol gradient and collected in each step of purification assay. The organic solvent was removed in a rotatory evaporator and the titre of the bacteriocin in the remaining aqueous solution was determined by the critical dilution assay, expressed as arbitrary units per ml (AU/ml), using *L. monocytogenes* as indicator strain. The final active fraction was concentrated by freeze-drying and partially purified bacteriocin was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to estimate molecular weight by means of silver staining and biological revelation of gel.

Results: Bacteriocin titre in initial *L. mesenteroides* A11 culture supernatant was 3,200 AU/ml. High recovery of bacteriocin was obtained with XAD-SPE since no loss was detected in bacteriocin quantification in fractions collected during purification steps. By SDS-PAGE, molecular weight of *L. mesenteroides* A11 bacteriocin was estimated as 3.5 to 8.5kDa.

Significance: Purification of antimicrobial peptides from *L. mesenteroides* A11 provided information that may be useful to design food biopreservation applications.

P3-78 Adhesion and Dispersion of *Listeria monocytogenes* on Abiotic Surfaces

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Introduction: Biofilms are aggregates of microbial cells attached to a surface, encased in a self-produced extracellular polymeric matrix. *Listeria monocytogenes* may form biofilms and persist in food processing environments, representing a threat for food safety.

Purpose: To study biofilms of *L. monocytogenes* using cultivation and microscopic techniques.

Methods: *L. monocytogenes* was inoculated (7 log CFU) in 24-wells polystyrene microplate containing sterilized stainless steel coupons (AISI 304, 1.57 cm^2) and BHI broth. It was incubated (25 °C, 72 h, 60 rpm) and adhered cells were enumerated every 24 h, by removal and washing of coupons, sonication, surface plating on BHI agar (37 °C/24 h) and counting. Biofilm formation was also evaluated by fluorescence microscopy using acridine orange to stain total DNA. Additionally, *L. monocytogenes* was cultivated (25 °C/192 h) in BHI broth in eight-chamber covered glass slide. At times 24, 96 and 192 h, biofilms were stained with LIVE/DEAD BacLight™ kit and observed with confocal laser microscope.

Results: Population of *L. monocytogenes* adhered to coupons after 72 h/25 °C was ca. 7 log CFU/cm^2. Under fluorescence microscopy, after 24 h, isolated cells and microcolonies of *L. monocytogenes* were observed adhered to the coupons, in the presence of extracellular matrix. Larger cell aggregates were seen at 48 h, while after 72 h there were channels and also and signs of dispersion (holes). Images of confocal microscopy of glass slides taken at 24 h, revealed a monolayer of viable bacterial cells and some hollows. At 96 h, the surface was patchy covered, but non-viable cells were abundant and voids were also observed. Several layers of viable cells covered the slide surface after 192 h, and holes of different sizes were observed.

Significance: *L. monocytogenes* form biofilms either in glass or stainless steel, and microscopic analysis revealed important aspects of biofilm architecture that may contribute to understand the persistence of the organism in nature.

P3-79 Microbiological Characterization of Unprocessed, Processed and Retail Samples of Commercial Brazilian Bee Pollen for Human Consumption

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Introduction: Commercially bee pollen is produced in specialized collectors and consists of pollen collected by honeybees and packed and mixed with nectar into granules which fall into a collection space. Currently in Brazil there are no specific regulations for microbiological parameters in bee pollen.

Purpose: The aim of this work was to evaluate the presence of potentially pathogenic bacteria, and yeast and molds in unprocessed, processed and commercially available bee pollen in Brazil.

Methods: Samples of bee pollen obtained from collectors at an experimental apiary in Pindamonhangaba, SP, Brazil, were collected at 24 hours, 48 hours and 72 hours intervals, to determine the change in potentially pathogenic microorganisms in relation to the time of production in the field. The samples were left unprocessed or were processed with standard methods in which they were first frozen for 48 hours at -18 °C, and then subjected to either dehumidification at 30 °C or air drying at 42 °C. The samples were evaluated for the presence of clostridia, coagulase-positive *Staphylococci*, coliforms at 45 °C, *Bacillus cereus*, *Escherichia coli*, *Salmonella*, and total molds and yeast counts. Samples, which had been registered at the Federal Inspection Service (SIF), were also acquired at retail markets in the state of Sao Paulo, Brazil, and were subject to the same evaluation.

Results: Of the unprocessed samples from the apiary, 100% contained coliforms, yeast and molds, 77% contained *Bacillus cereus* and 33% clostridia. In all of the processed samples there was a reduction in the levels of bacteria, however, high levels of yeast and molds remained (in comparison to allowed levels in other commercial foods such as pasta and flour). In the samples acquired from the markets, 70% contained *Bacillus cereus*, 60% had relatively high yeast and mold counts (> 10^3 CFU/g), 40% contained clostridia and 10% contained coliforms.

Significance: These data demonstrate bee pollen can contain potentially pathogenic bacteria as well as high yeast and mold counts and the need for a greater understanding of its microbial ecology with regard to human health for use in improving regulatory standards.

P3-80 Probiotic Potential of Lactic Acid Bacteria Isolated from Fermented Greek Table Olives

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Introduction: Table olives are one of the most important traditional fermented food and a basic component of the Mediterranean diet. The great nutritional value of table olives, in conjunction with their flavor and taste, has been well established.

Purpose: To evaluate the probiotic potential of strains isolated from olives to be used as starters for the improvement of the traditional fermentation and the production of a new functional food.

Methods: Seventy-one lactic acid bacterial strains (isolated from olives) were screened for their probiotic potential, following a series of *in vitro* tests. 17 strains were *Leuconostoc mesenteroides*, 1 *Ln. pseudomesenteroides,* 13 *Lactobacillusplantarum,* 37 *Lb. pentosus,* 1 *Lb. paraplantarum*, and 2 *Lb. paracasei subsp. paracasei*. *Lb. rhamnosus* GG and *Lb. casei* Shirota were used as reference strains. All isolates were tested for their survival in simulated gastrointestinal tract conditions, antimicrobial activity (against *Listeria monocytogenes, Salmonella* Enteritidis*, Escherichia coli* O157:H7), resistance to 9 antibiotics, Caco-2 surface adhesion and hemolytic activity.

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Results: Three *Lb. pentosus* strains, 4 *Lb. plantarum* strains and 2 *Lb. paracasei subsp. paracasei* demonstrated the highest populations (≥ 8 log CFU/ml) after 3 h of exposure at low pH. The majority of the tested strains were resistant to bile salts even after 4 h of exposure. 5 *Lb. plantarum* and 7 *Lb. pentosus* strains exhibited partial bile salt hydrolase activity. None of the examined strains exhibited *β*-hemolytic activity. Variable susceptibility of the strains towards the different antibiotics and their efficiency to adhere to Caco-2 cells was observed. None of the tested strains inhibited the growth of the pathogens.

Significance: The fact that some of the tested strains possess probiotic properties enhances their potential of being used as starter cultures to produce a traditional product with desirable fermentation characteristics and added value.

P3-81 The Effects of Aging Times and Temperatures on *Bacillus cereus* Spores Survival in Wet Noodles during Cooking

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Developing Scientist Competitor

Introduction: The consumption of wet noodles has frequently been questioned about the potential of food poisoning due to *B. cereus* spores, which may germinate, grow, and survive even after cooking.

Purpose: The purpose of this study was to evaluate the survival characteristics of *B. cereus* spores throughout the noodle manufacturing steps and cooking.

Methods: *B. cereus* spore cocktail of 5 strains was added when preparing dough, and resulted in 5 log CFU/g concentration. The dough was aged for 0, 1, 2, 4, 6 and 8 hours at 20, 25, 30, 35 and 40 °C. The number of spores was estimated at each manufacturing step and the survival of spores was evaluated with times (2, 4, 6, 8, 10 and 12 min) while the noodles were cooked at boiling water.

Results: The number of *B. cereus* was not significantly different with aging times and temperatures, which indicates no growth in wet noodle environment even though it may germinate. All spores in wet noodles without aging were destroyed after 12 min cooking and $D_{110°C}$ value was found as 1.8 min. After 1 hour aging, the cooking time was shortened to 10 min at 20 °C and reduced by 2 min with increase of aging temperature by 10 °C. This means that the higher aging temperature induces more germination of spores and results in faster destruction of *B. cereus* in wet noodles.

Significance: These results suggest that higher temperature may be applied for wet noodle aging to kill *B. cereus* faster during cooking and thus may not pose the risk of food poisoning.

P3-82 Tracking of Antibiotic Resistant Gene Transfer from a Known Donor to Unknown Recipients in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME)

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Developing Scientist Competitor

Introduction: Transfer of antimicrobial resistant genes from contaminated food to the resident microbiota of the GI tract may pose a clinical concern. Knowing which are the donors and/or recipients involved in this gene transfer is problematic as the gut microbiota consists of 500-1,000 species of bacteria. Thus, to determine the potential bacteria capable of horizontal gene transfer in this environment is of interest.

Purpose: The present study employed the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) as a model to evaluate gene transfer from a known donor to unknown recipients in fecal culture pool.

Methods: The SHIME system was set up following Van den Abbeele et al. (2010) with modification. *E. coli* O157:H7 carrying a plasmid containing *lux* gene and kanamycin and ampicillin resistant genes was introduced into the SHIME. Selective pressure (kan, 50 µg/ml) was applied to induce horizontal gene transfer among the microbes. Luminescence was used to track gene transfer.

Results: An unknown luminescent bacterium was isolated and identified as *Pseudomonas aeruginosa* using the Riboprinter. PCR and restriction analysis of bacterial DNA confirmed the presence of the *lux* gene and the same plasmid as in the *E. coli* O157:H7 donor.

Significance: This study employed luminescence as an indicator to track antibiotic resistance gene transfer among resident gut flora. In addition, our study also established a direct relationship between gene donor and gene recipient in the simulated gut model.

P3-83 Validation of the Use of Composite Sampling for the Detection of *Listeria monocytogenes* in Frozen Vegetables

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Introduction: Multiple methods have been validated to detect *Listeria monocytogenes* in small samples (25 g) of frozen vegetables. Little work has been done to determine if these methods are effective at detecting low levels of *Listeria monocytogenes* in 125 g and 375 g composited frozen vegetable samples. In this study, the U.S. Food and Drug Administration's Bacteriological Analytical Manual (Online Edition), enzyme-linked fluorescent immunoassay (ELFA) and polymerase chain reaction (PCR) methods were used to evaluate the efficacy of detecting low and medium levels of *Listeria monocytogenes* in composited frozen samples of broccoli, tomato, potato and onion.

Purpose: The purpose of this study was to evaluate the efficacy of compositing frozen vegetables using multiple detection methods for *Listeria monocytogenes* at low and medium inoculation levels.

Methods: A four-strain *Listeria monocytogenes* cocktail was used to spike 25 g, 125 g and 375 g samples of frozen vegetables at low and medium levels. Samples were enriched for each test method with volumes of pre-enrichment modified to accommodate the 125 g and 375 g samples sizes. Data were analyzed using the chi squared test to determine statistical significance.

Results: At the medium inoculation level, *Listeria monocytogenes* was detected by all methods in all 25 g, 125 g and 375 g samples. When considering all vegetables and methods using the low inoculation level, the ability to detect *Listeria monocytogenes* in the 375 g samples was significantly lower than the 25 g samples.

Significance: This study provides data for evaluating the use of sample compositing of frozen vegetables using multiple methods of detection for *Listeria monocytogenes*.

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P3-84 Preservation of Industrially Important Microorganisms

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Introduction: Although thousands of microorganisms have been described in the literature, microbiologists often lose cultures because of the absence of reliable culture collections. Pathogens that are linked to food/food-animals and their control are a main concern of the industry and producers. Furthermore, it is of interest of the poultry and swine industries to have a reliable collection to support research and product quality improvement.

Purpose: Brazil has one of the most developed poultry industries of the world and is an important swine producer/exporter. Therefore, it is essential to have a depository of reference strains for future research. The microbial collection (CMISEA) of Embrapa Swine and Poultry is recognized as a trusted depository, which can potentially store over 12,000 microorganisms. CMISEA's main goal is to maintain pathogenically important microorganisms for the poultry/swine industries, serving as a reliable depository of properly preserved cultures.

Methods: Currently, the laboratory is equipped with liquid nitrogen tanks, ultrafreezers, freezers, refrigerators, cabinets designed for room temperature storage and a lyophilizer. All isolates are stored in sextuplicates in at least two different temperatures.

Results: Currently, 99% of the isolates deposited are bacteria and 1% are viruses. In addition, 86% were isolated from swine sources and 14% from poultry samples. The collection holds 491 different microorganisms within sixteen genera. The most representative is *Pasteurella* (26%), then *Bordetella* (23%) and *Salmonella* (18%). The laboratory has also a bacterial-viral library with more than 8,000 isolates and the most representatives are *Salmonella* (44%), *Escherichia* (16%), *Infectious bronchitis virus* (21%) and *Suid herpesvirus-1* (20%).

Significance: The microorganisms deposited in the CMISEA are of importance for the poultry and swine industries, as well as for academia and research. It is strategic for the country to have a collection for future studies, such as, pathogen characterization and prevalence. Other applications are outbreak control, bioprospecting and genomic characterization.

P3-85 *Listeria innocua* Hinders Recovery of *Listeria monocytogenes* Serotype 4b during Selective Enrichment in Buffered *Listeria* Enrichment Broth

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Introduction: Selective enrichment procedures are typically used to increase the population of *Listeria monocytogenes* while inhibiting the growth of additional non-pathogenic microorganisms that might be present in the test sample. Enrichment broth formulations rarely demonstrate absolute target organism selectivity. It is possible that when more than one species of *Listeria* are present in a food sample the non-pathogenic species could be preferentially recovered.

Purpose: In order to gauge the extent of preferential recovery during the selective enrichment procedure, ten isolates each of *L. monocytogenes* and *L. innocua* were cultured together under selective conditions for a total of 100 isolate pairings.

Methods: Hemolytic patterns on blood agar plates were used to obtain presumptive 48 hour populations of each species. Species confirmation was performed by conventional PCR. Isolated colonies were also obtained using the streak plating method on a variety of selective and differential media. Ten typical colonies were selected from each plate for species confirmation.

Results: The initial inoculum ranged from 1.3 to 2.4 log CFU/mL with a mean of 2.2 ± 0.3 for *L. innocua* and ranged from 1.7 to 2.6 log CFU/mL with a mean of 2.1 ± 0.1 for *L. monocytogenes*. The final population of *L. monocytogenes* range from 7.0 to 9.0 log CFU/mL with a mean of 7.8 ± 0.6 and for *L. innocua* ranged from 8.3 to 9.7 with a mean of 9.2 ± 0.2. In 98 of 100 isolate pairings, *L. innocua* reached higher levels than did *L. monocytogenes* with a difference ranging from 0.2 to 2.4 logs and a mean log difference of 1.5 ± 0.6. Species confirmation following colony selection from non-differential media confirmed the preferential recovery of *L. innocua* from 48-hour enrichments.

Significance: The presence of non-pathogenic *Listeria* species may be indicative of low levels of *L. monocytogenes* which can go undetected with traditional streak plate procedures. Additional alternative testing should be used to minimize false negative sample reporting.

P3-86 Endemic *Salmonella* Contamination of the Virginian Eastern Shore Tomato Production Environment

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Introduction: Non-typhoidal *Salmonella* species are responsible for an estimated 1 million foodborne illnesses every year in the U. S. Tomatoes grown along the Eastern Shore of Virginia are implicated almost yearly in *Salmonella*outbreaks.

Purpose: The results of a four-month survey conducted in the summer of 2011 for environmental *Salmonella* on Virginia's Eastern Shore (VES) are presented.

Methods: Samples were collected at Virginia Tech Agricultural Research Extension Center from June to September. To ascertain the prevalence of *Salmonella* in VES waterways, six additional creeks were sampled. For each collection, 84 to 255 samples of soil, sediment, water, insects, produce and vegetation were enriched in modified buffered peptone water and tested for *Salmonella* using a multiplex, real-time PCR (qPCR) assay. The qPCR tested for the *Salmonella* specific markers, *invA*and *apeE* along with *gapA*, a marker for *Enterobacteriaceae*, and an internal amplification control. Subsets of samples were also cultured for isolation of *Salmonella*.

Results: The incidence of *Salmonella* detection via the real-time PCR method was the highest in September. These samples were collected after Hurricane Irene and do not follow the trend of detection seen in previous years. Data from June to August do follow the previous trend showing the highest incidence in June and decreasing each subsequent month. Over 200 isolates, 17+ serovars including Newport and Javiana, were recovered. The majority of these isolates came from the various waterways along the VES. Also the most serotype diversity was observed in the isolates from VES waterways.

Significance: This suggests that *Salmonella* has established itself as an environmental resident along the VES. Several isolates were obtained from goose feces suggesting a possible mechanism of spread of *Salmonella* throughout this environment. Many of these isolates are undergoing whole genome sequencing to gain a greater understanding of this resident environmental population.

P3-87 Isolation of *Bacillus cereus* from Pineapple Pulp and Partial Characterization of a Bacteriocin-like Substance

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Introduction: Food additives are widely used in processed foods and beverages, but the safety of many of these compounds is a matter of debate and, the bacteriocins constitute a potential alternative of less toxic preservatives for use in food biopreservation.

Purpose: To identify a *Bacillus* sp. isolate from pineapple pulp that produces a bacteriocin-like substance (BLIS) and to purify partially the antimicrobial peptide.

Methods: *Bacillus* sp. was isolated on MRS agar, cultured in MRS broth (24 h/30 °C) and, its activity against *Listeria monocytogenes* was tested by critical dilution assay (AU/ml). The neutralized filter-sterilized supernatant of the MRS culture was purified with Amberlite® XAD-16 resin (Sigma-Aldrich) using water, 20% ethanol and 70% isopropanol. Evaporated extract was applied on solid phase extraction column - SPE (C18, Varian) and eluted with water, 30% ethanol and 20, 40 and 70% isopropanol. The last fraction was selected, solvents were removed and the extract was analyzed by tricine SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Gel was revealed by silver staining and with *Listeria monocytogenes* (biological revelation). Possible hemolytic activity of extract was tested by streaking it on blood agar plate (37 °C/24 h).

Results: Isolate was named *Bacillus cereus* LFB – FIOCRUZ 1638 and the initial supernatant presented activity of 400 AU/ml. The antimicrobial activity of supernatant was stable at 75 °C/30 min, but 50% of activity was lost at 95 °C/30 min, while no activity remained after 121 °C/20 min (autoclave). Activity was detected also in supernatant when pH varied from 1.9 to 8.0 (10 °C for 24 h). The molecular weight of the BLIS was estimated by SDS-PAGE as 3.5 to 8.5kDa.

Significance: Despite negative association of *Bacillus cereus* with foods, purified bacteriocin preparations from *Bacillus* sp. may have technological advantages for food processing when compared to bacteriocins from lactic acid bacteria, due to large thermal and pH stability.

P3-88 Safety of Bacteriocinogenic Strains Isolated from Traditional Smoked Meat Products from North Portugal

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Introduction: LAB capable of producing bacteriocins have technological applications in smoked meat products' biopreservation. However, presence of virulences factors, antibiotic resistance or biogenic amine genes are critical in determining the safety aspects for LAB.

Purpose: To screen for the presence of genes encoding bacteriocin, virulence factors, antibiotic resistance and biogenic amines in LAB able to produce antimicrobial compounds, isolated from smoked meat products.

Methods: The microbiological status (presence of *Listeria* spp., *Aeromonas* spp., LAB and total microbial count) of 43 industrial smoked and fermented meat products from Portugal, produced without starter cultures, was studied. Isolated LAB were identified by 16s rDNA sequencing and tested for the production of bacteriocins against various *Listeria monocytogenes* strains and screened by PCR for genes encoding bacteriocins, virulence factors, antibiotic resistance and biogenic amines formation.

Results: The microbiological status of tested meat products showed that fermentation was dominated by LAB (10^7 CFU/g), consisting of "wild" strains of *Lactobacillus* spp. and *Enterococcus* spp. The presence of *L. innocua* in tested products was recorded in 32 of 43 samples. *L. monocytogenes*, *L. ivanovii* and *Aeromonas* spp. were not detected in any of the samples. Six bacteriocinogenic strains (2 strains of *Lactobacillus plantarum*, 3 strains of *Lb. sakei* and 1 strain of *Enterococcus faecium*) were selected and tested for production of bacteriocins against *L. monocytogenes* from different serological groups. No evidences for presence of genes for ornithine decarboxylase, hystidine decarboxylase, tyrosine decarboxylase, gelatinase, aggregation substance, cytolisin, endocarditis antigen and vancomycin A were recorded in all tested strains. Vancomycin B gene was detected in *Lb. sakei* ST153Ch and *Lb. plantarum* ST216Ch; adhesion of collagen protein gene generated positive results for *Lb. sakei* ST22Ch and ST153Ch and *Lb. plantarum* ST216Ch; enterococcal surface protein and hyaluronidase were present in *Lb. sakei* ST154Ch.

Significance: *Lb. plantarum* ST202Ch and ST216Ch, *Lb. sakei* ST22Ch, ST153Ch and ST154Ch and *E. faecium* ST211Ch produce antilisterial bacteriocins, harbored at least one bacteriocin gene and presented low levels of presence of virulence genes. These strains present interesting potential for technological applications in the meat industry.

P3-89 Efficacy of Sanitizers Approved for Organic Use against *Salmonella enterica* on Organic Leafy Greens

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Introduction: Consumption of organic produce has been increasing in recent years. *Salmonella* is one of the pathogens of concern for the produce industry because of its association with foodborne illness outbreaks from fresh produce. Post-harvest sanitizing is an effective method to reduce microbial contamination in fresh produce.

Purpose: The objective of this study was to compare the efficacy of various sanitizers approved for organic use against *Salmonella* Newport on four types of organic leafy greens−Romaine and iceberg lettuce, and mature and baby spinach.

Methods: The sanitizers tested included 3% hydrogen peroxide, formulations of chlorine dioxide (CD), calcium hypochlorite (CH), and a mixture of peroxyacetic acid and hydrogen peroxide (PAH). The concentrations of the working solutions were determined according to the manufacturer's directions (CD at 11 ppm, CH at 100 and 200 ppm, and PAH at 85 and 200 ppm). Water was used as a control. Leafy green samples were washed and dip inoculated in 10^6 CFU/ml*Salmonella* Newport culture. Samples were dried in biohood for 1 hour, and then immersed in various concentrations of sanitizers for 2 min. After storage at 4 °C for 0, 1 and 3 days, samples were taken for enumeration of survivors.

Results: After sanitizer treatments, there were 0.5-1.6 log additional reductions of *Salmonella* compared to water treatment ($P < 0.05$) at day 0. The sanitizer solutions containing 200 ppm PAH and 200 ppm CH showed the best reductions on all types of organic leafy greens at day 0 (0.9-1.6 logs). There was a slight increase (<0.4 logs) in reductions of *Salmonella* population by day 1 and 3, for both water and sanitizer treatments. In general, the sanitizer treatments were more effective on iceberg lettuce than on other leafy greens.

Significance: The results from this study could provide organic leafy green industry with more options to select sanitizers for post-harvest treatments.

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P3-90 Enhanced Plasmid Transformation of *Campylobacter jejuni* NCTC11168 through Cj1051c Mutagenesis

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Introduction: *Campylobacter jejuni* is an important human enteric pathogen; however, general knowledge and available tools for this bacterium are lacking compared to other enteric pathogens. *C. jejuni* strain NCTC11168 is a common laboratory strain and the first *C. jejuni* strain to be sequenced. The apparent inability to transform plasmids into this strain complicates, and sometimes inhibits, its use.

Purpose: We attempted to isolate genetic factors which may contribute to the difficulties in plasmid transformation of *C. jejuni* NCTC11168. Mutagenesis of these factors may yield a strain which can facilitate further research of *C. jejuni*.

Methods: Random transposon mutants of NCTC11168 were electroporated with the green fluorescent protein (GFP) expressing shuttle plasmid pWM1007 and transposon insertion sites determined by plasmid rescue. Knock out mutagenesis of Cj1051c was performed by overlapping extension PCR creating a construct with the genomic regions flanking Cj1051c cloned around chloramphenicol or tetracycline resistance cassettes followed by electroporation into *C. jejuni*NCTC11168.

Results: The transposon screen produced 164 colonies which appeared to have successfully taken up the plasmid. Plasmid rescue of two isolates determined the transposon insertion to be in Cj1051c, a gene predicted to be part of a restriction modification system. Knock out strain *C. jejuni* NCTC11168DCj1051c was found to have an enhanced transformation rate of >600 colonies per microgram of pWM1007 while the wild-type strain had sporadic transformation success with few to no colonies produced from a microgram of pWM1007.

Significance: Strain NCTC11168DCj1051c may be a useful tool in molecular biology research of *C. jejuni* as it allows enhanced plasmid transformation. The parental strain is one of the most commonly used strains for genetic studies and this mutant will facilitate research that had previously been difficult or impossible without genomic disruptions.

P3-91 Development of Antimicrobial Surface-modified Stainless Steel with N-halamines: Characterization and Effectiveness against *Listeria monocytogenes*

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Introduction: Cross-contamination of pathogenic bacteria from contaminated food processing surfaces poses a significant risk to food safety. Stainless steel is commonly used to fabricate food processing equipment and has been shown to harbor pathogens like *Listeria monocytogenes*. Development of antimicrobial stainless steel in which bulk material properties are retained and the antimicrobial activity is long-lasting can support current cleaning and sanitization efforts to improve food safety. N-halamines represent a unique class of antimicrobials capable of recharging their antimicrobial activity with each exposure to halogen-based sanitizers.

Purpose: The purpose of this study was to modify the surface of stainless steel to possess rechargeably antimicrobial N-halamines, to characterize the changes in surface chemistry and finally to evaluate the antimicrobial activity of the modified steel against *Listeria monocytogenes*.

Methods: Surface modification of stainless steel was performed via layer-by-layer deposition of N-halamine containing polymers and different types of analysis were employed to confirm its chemical modification: FTIR, ellipsometry, X-ray photoelectron spectroscopy, acid orange 7 assay and contact angle. Antimicrobial activity against *Listeria monocytogenes* was performed by submersion of control and modified steel in bacterial suspensions under varying conditions.

Results: The reported layer-by-layer surface modification technique effectively introduced antimicrobial N-halamines to stainless steel. Primary amines increased significantly with each N-halamine multilayer. At six multilayers, the stainless steel presented 36.8 ± 6 nmol/cm^2 of antimicrobial N-halamines. A single multilayer of polymers added onto the surface had a thickness of only 2.0 ± 0.2 nm. The effectiveness against *Listeria monocytogenes* was demonstrated, in which N-halamine modified steel was able to inactivate 5 – 6 log CFU/ml in less than 1 h.

Significance: Results indicate that our N-halamine modified stainless steel effectively inactivates *Listeria monocytogenes* and may therefore represent a means to control the cross-contamination of pathogens from food processing surfaces, reducing the risks of foodborne illness.

P3-92 Impact of Post-inoculation Hold Time when Treating *Escherichia coli* O157:H7- and *Salmonella*-inoculated Lettuce and Tomatoes with Chlorine Dioxide Gas

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Introduction: Chlorine dioxide (ClO_2) gas is gaining popularity as an antimicrobial agent for fresh and fresh-cut produce.

Purpose: This study assessed the impact of post-inoculation hold time on antimicrobial efficacy of ClO_2, against the two bacterial pathogens most often responsible for fresh produce-related outbreaks.

Methods: Three batches of fresh-cut Romaine lettuce and cherry tomatoes were, respectively, dip-inoculated with 3- and 2-strain cocktails of *Escherichia coli* O157:H7 (K3995, K4492, K4830) and *Salmonella* spp. (*S.* Montevideo MDD22, *S.* Newport MD313) to contain ~7.90 log CFU/g and then held for 1 and 24 h before gassing. Both products were exposed to ClO_2 gas generated from a MiniDox-M (ClorDiSys, Lebanon, NJ) at 3, 6 or 10 mg/l for up to 30 min. Thereafter, *E. coli*O157:H7 and *Salmonella* populations were, respectively, quantified in 25-g samples by direct plating appropriately diluted rinse solutions on trypticase soy agar (TSA) overlaid with sorbitol MacConkey agar supplemented with cefixime and potassium tellurite and TSA overlaid with xylose lysine deoxycholate agar.

Results: Holding inoculated lettuce for 1 and 24 h before treatment yielded similar *E. coli* O157:H7 reductions of 1.53 ± 0.08 and 1.48 ± 0.14 log CFU/g after 30 min of exposure, respectively. However, ClO_2 gassing was significantly less efficacious ($P < 0.05$) for tomatoes at the longer post-inoculation hold time with *Salmonella* populations on 1- and 24 h- held tomatoes decreasing up to 7.19 ± 0.00 and 5.81 ± 0.13 log CFU/g, respectively. As expected, a significant interaction ($P < 0.0001$) was seen between ClO_2 concentration and treatment time (concentration*time) for both lettuce and tomatoes.

Significance: When evaluating the efficacy of ClO_2 for field-grown tomatoes, post-inoculation hold times of at least 24 h should be considered so as to not overestimate the effectiveness of ClO_2 against foodborne pathogens.

P3-93 A Comparison of the *in vitro* Anti-microbial Effectiveness of Different Organic Acids and Salt Derivatives against Two *Listeria monocytogenes* Strains

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Introduction: The food sector has seen emerging cases of foodborne outbreaks caused by *Listeria monocytogenes* while the organism has been becoming increasingly resistant to conventional antimicrobials and preservatives.

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Purpose: This article reports on the anti-listerial properties of selected organic acids and salt derivatives in order to inform possible alternatives in food preservation and pathogen control.

Methods: The susceptibility of two *Listeria monocytogenes* strains was assessed against five organic acids and two acid-salt derivatives across a series of pH environments. Minimum inhibitory concentrations (MICs) of the acids were tested against the two strains by means of an agar-dilution method

Results: In general, ATCC 19117 was found to be more resistant to both organic acids and salts. High MIC levels (low susceptibility) were noted for potassium sorbate, sodium benzoate and lactic acids, while at pH 5 the isolates were susceptible to all the organic acids tested. A small increase in pH notably reduced antimicrobial activity against the organisms. At pH 7 the isolates all but lost susceptibility to benzoic, lactic, malic and sorbic acids. Although the activity of the majority of acids was linked to pH, some acids were not as closely related (e.g., potassium sorbate, sodium benzoate and citric acid) and this suggests that the type of organic acids plays a role in inhibition. The relatively high MICs reported for compounds that are conventionally used as preservatives against *Listeria* spp. raises concern.

Significance: The results suggest that the type of organic acid used to set pH, and not only pH alone, plays a role in determining inhibition. It was confirmed that a "one-size-fits-all" approach to preservation is not always effective. Furthermore, the need for microbiological data to sub-species level to inform the selection of preservatives was highlighted.

P3-94 *Withdrawn*

P3-95 Fate of *Listeria monocytogenes* during the Maturation of Salami Containing Encapsulated Bacteriocin-producing *Lactobacillus curvatus* MBSa2

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Introduction: *Listeria monocytogenes* (LM) is a pathogen able to survive several environmental stresses. One strategy to prevent the growth of LM in meat products can be the addition of bacteriocin-producing lactic acid bacteria (LAB). One of the main problems concerning of the utilization of such LAB in these products is the low yield of bacteriocin production. It is known that immobilization of LAB can keep the microbial concentration and improve the production of lactic acid. However, little is known about its influence *in situ* in the bacteriocin productio.

Purpose: This study aimed at evaluating the behavior of *Listeria monocytogenes* during the maturation of salami added of alginate-encapsulated *Lactobacillus curvatus* MBSa2, a bacteriocin-producer LAB isolated from salami.

Methods: MBSa2 strain was cultured in MRS broth, harvested by centrifugation and washed with peptone water (0.1%). The cells were encapsulated by adding 2% alginate and dripping the mixture in 100 mM $CaCl_2$ solution. Salami was produced mixing pork meat, beef meat, fat, salt, Compact salami 160 (Kraki), starter culture T-SPX (Christian Hansen) and free or encapsulated *L. curvatus* MBSa2, and experimentally contaminated with LM (10^4-10^5 CFU/ml). Controls, added of a non-bacteriocin producing LAB (*Lactobacillus sakei* ATCC 15521) were also prepared. Meat mixture was fermented for four days (99%-97% RH, 20 °C) and ripened for 26 days (97%-75% RH, 18° - 15 °C). Counts of viable LAB and LM and determinations of the level of bacteriocin produced, pH and A_w were performed after 4, 10, 20 and 30 days.

Results: After of 30 days of maturation, counts of *L. monocytogenes* in salami with free *L. curvatus* MBSa2, encapsulated *L. curvatus* MBSa2, free *L. sakei* ATCC 15521 and with no added LAB were reduced 1.95, 2.47, 1.65 and 2.00 log CFU/g, respectively. No bacteriocin production was detected in all conditions studied, during 30 days of maturation. Initial pH values of the meat product were 5.97-5.92, and were reduced to 5.23-5.15 in 4 days, reaching 5.52-5.38 in 30 days. Initial A_w values were 0.98-0.97 and were reduced to 0.91-0.88 in 30 days.

Significance: These results indicate that reduction of counts of LM during the maturation of salami was similar in the tested products, regardless the presence of free or encapsulated bacteriocinogenic *L. curvatus* MBSa2.

P3-96 Vapor-phase Antimycotic Activity of *Lippia berlandieri* and *Poliomintha longiflora* Essential Oils

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Introduction: Spices and their essential oils (EOs) have antimicrobial properties, which justifies its addition in processed foods, either directly or exposed to their vapors.

Purpose: The antifungal activity in vapor-phase of two Mexican oregano EOs (*Lippia berlandieri* Schauer or *Poliomintha longiflora*) on the growth of *Aspergillus flavus* was assessed in a model system, at selected temperatures and concentrations.

Methods: EOs were obtained by steam distillation, characterized by determining physical properties such as density, refractive index, and color, and their chemical composition by GC-MS. Potato-dextrose agar plates were center inoculated pouring 2μL of *A. flavus* spore suspension. Inoculated plates were incubated at 25, 30, or 35 °C in 1.7 l airtight chambers, in which plates without lids were placed with selected amounts of each studied EO to achieve concentrations of 14.7, 29.4, 58.8, or 117.6 μl / l of air, as well as a control (without EO). During incubation, colony diameters were measured. *A. flavus* radial growth rate and lag phase in the presence of vapors of each EO at studied temperatures were calculated. The effect of EO concentration and temperature on growth parameters was evaluated by polynomial equations. Further, minimum inhibitory concentrations (MIC) of EOs at different temperatures in which the effect was fungistatic or fungicidal were determined.

Results: Incubation temperature as well as EO concentration exerted significant effects ($P < 0.05$) on growth rate and lag phase of *A. flavus*; at higher concentration of EOs and lower temperatures, growth rate decreased and lag phase increased, suggesting that tested EOs act by delaying or inhibiting mold germination stage. *P. longiflora* EO was significantly ($P < 0.05$) more inhibitory than *L. berlandieri* EO, which can be attributed to differences in composition of volatile compounds with antimicrobial activity. Carvacrol: thymol ratio in *L. berlandieri* EO was 12:1 while in *P. longiflora* EO was 1:3.

Significance: Mexican oregano EOs applied in vapor-phase exert significant antifungal activity.

P3-97 Determination of the Minimal Inhibitory Concentration of Lauric Arginate against Three Strains of *Salmonella enterica*

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Developing Scientist Competitor

Introduction: Lauric arginate (LAE) is a food grade cationic surfactant derived from the integration of lauric acid, L-arginine, and ethanol. Although it possesses antimicrobial capabilities, its commercial application in food and beverage systems is currently limited due to its bitter/

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astringent taste and low levels of solubility.

Purpose: The objective of this study was to determine the minimum inhibitory concentration of LAE on *Salmonella* in a broth system.

Methods: To determine the minimum inhibitory concentration (MIC) of LAE in a broth system, LAE (5%, pH 7.0, 10 mM Phosphate Buffer) stock solution was diluted to varying ppm concentrations (0, 1, 2, 4, 8, 16, 32, 64, 128, and 256) and randomly distributed in microtiter plates. A 1% inoculum of *Salmonella enterica* (ATCC strains BAA-708, BAA-709, and BAA 710) overnight culture was added to 2X TSB, combined with equal amounts of LAE and incubated at 32 °C and 20 °C. After 6, 24, 48, and 72 hours of incubation, O.D. at 570 nm was measured. The MIC was defined as the lowest concentration that inhibited bacterial growth (as measured by OD) after 72 h. Experiments were performed with three independent replicates.

Results: The minimum inhibitory concentration of LAE in a broth system was dependent on strain but not incubation temperature. At both temperatures, strains BAA-708 and BAA-709 were inhibited at 256 ppm, while growth of strain BAA-710 was inhibited at 128 ppm. These results indicate that the incorporation of LAE in a concentration of 256 ppm would likely be effective in inhibiting *Salmonella enterica* in other systems.

Significance: By determining the MIC against multiple *Salmonella enterica* strains, the most appropriate amount of LAE can be selected for use in food and beverage products.

P3-98 Antiviral Effects of Cell-free Bacterial Supernatants

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Introduction: Bacterial extracellular proteases are known to contribute towards antibacterial properties with their range of targets generally recognized as specific and narrow; however, bacterial excretions with broad antibacterial and even antiviral effects have been reported.

Purpose: This study was undertaken to evaluate the effect of cell-free bacterial supernatants on the cytopathic effect of murine norovirus on RAW cells.

Methods: *Enterococcus faecalis* was grown in TSB, and a commercial probiotic mixture (*Lactobacillus acidophilus*, *L. rhamnosus*, *Bifidobacterium bifidum*, *L. salivarius*, and *S. thermophilus*) was grown in UHT-pasteurized milk. Cell-free supernatants (CFS) were prepared by centrifugation of the bacterial suspensions and filtration of the supernatants through 0.2-µm pore filters. The cytopathic effects of 10^4 infectious MNV on RAW cells was evaluated in the presence of 10% CFS in DMEM or after RAW cells were exposed to CFS and repeatedly washed with HBSS. Cytopathic and cytotoxic effects were evaluated by microscopic examination 48 h post inoculation. At 10% in DMEM, HBSS, TSB, milk, and milk acidified with lactic acid not inoculated with bacteria were included as controls.

Results: Cytopathic effect of MNV was reduced in the presence of CFS of *E. faecalis* as compared to controls and was inconsistent in the presence of the CFS of the probiotic mix. No cytopathic effect of MNV on RAW cells was observed in the continuous presence of acidified milk or filtered supernatant of acidified milk or even after washing cells of these solutions prior to infection with MNV. Cytopathicity of MNV was not inhibited in cells washed of CFS. The effects were not pH-dependent. There were no apparent cytotoxic effects of the solutions. Challenges were encountered in quantifying the inhibition of viral infection by quantitative reverse-transcriptase PCR.

Significance: Varying degrees of inhibition of viral cytopathic effects were observed with continuous exposure to bacterial CFS; however, the effect was no greater than that observed with acidified milk controls. The nature of viral inhibition may be due to effects on the host cell and remains to be elucidated.

P3-99 Inhibition of Foodborne Pathogens and Spoilage Organisms in Dairy Dessert and Tomato Sauce Using Potassium Cinnamate

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Introduction: Potassium cinnamate has excellent antimicrobial properties. *Listeria monocytogenes* has a potential for growth in dairy dessert and *Zygosaccharomyces bailii* is a preservative-resistant spoilage microorganism associated with tomato sauce. In the present work, potassium cinnamate is evaluated on antimicrobial efficacy against pathogenic and spoilage microorganisms in dairy dessert and tomato sauce.

Purpose: The objective is to evaluate the antimicrobial efficacy of potassium cinnamate against *L. monocytogenes* in dairy dessert and *Z. bailii* in tomato sauce.

Methods: *L. monocytogenes* was inoculated to have ca. 2-3 log CFU/ml counts in the dairy dessert with pH of 6.38, and a_w of 0.992. Treatments with potassium cinnamate (Pura**Q**™ Xtend AX66) with 0, 0.10, 0.20, and 0.30% concentrations were prepared. The *L. monocytogenes* inoculated samples were stored at 4 °C for analyses throughout the incubation period. *Z. bailii* was inoculated to have ca. 2-3 log CFU/ml counts in the tomato sauce with pH of 3.87, and a_w of 0.940, respectively. Treatments with potassium cinnamate (Pura**Q**™ Xtend AX66) with 0, 0.02, 0.06, and 0.10% concentrations were prepared. The *Z. bailii* inoculated samples were stored at 12 °C for analyses throughout the incubation period. The plating for each microorganism was done independently at ca. 0, 3, 7, 14, 21, and 42 days.

Results: The *L. monocytogenes* growth data in the dairy dessert indicate that at minimum use level of 0.1%, potassium cinnamate arrested the growth of the pathogen to the inoculation levels over the incubation period. The tomato sauce study for*Z. bailii* indicate that at 0.02% use level, potassium cinnamate controlled the growth for 14 days and at 0.06% concentration levels, the growth of *Z. bailii* was inhibited over the incubation period.

Significance: This research substantiates the excellent antimicrobial efficacy of potassium cinnamate against pathogenic and spoilage microorganisms at very low levels in dairy dessert and tomato sauce. This research provides food industry with a highly effective antimicrobial to assure the food safety as well as to extend the shelf-life of food products.

P3-100 Antifungal Activity of Orange Peel Essential Oil Applied by Direct Addition or Vapor Exposure

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Introduction: The antifungal activity of orange essential oil (EO) has been scarcely studied, although it is claimed (using the agar dilution technique) that it is effective against the growth of some molds.

Purpose: The effectiveness of orange peel EO to control *Aspergillus flavus* growth was evaluated, comparing the antimicrobial efficacy of vapor exposure with EO direct addition.

Methods: EO was extracted from fresh orange peel by steam distillation with a yield of 0.5%. This EO had a density of 850 kg/m³, a refractive index of 1.47, and Hunter color coordinates L, *a*, and *b* that described a clear liquid with a slight shade of orange. The main compounds identified in the orange peel oil by gas chromatography coupled with mass spectrometry were: limonene, β-myrcene, β-pinene, α-pinene, and citral Z and E; of which, limonene represented a 96.62 %. Orange peel oil was applied to model systems (potato-dextrose agar) inoculated with *A. flavus*, using

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the techniques of direct addition to the agar or the generation of EO vapors in airtight containers where inoculated plates were also placed. Inoculated plates were incubated at 25 °C for 30 days. Every day mold colony diameters were measured; with these data and using Gompertz equation, radial growth rate and lag phase were calculated.

Results: In both methods *A. flavus* growth decreased when increasing EO concentration. Furthermore, although the effect of EO direct addition was faster, orange peel EO vapors were more effective, since lower concentrations were required to achieve the same antifungal effect. The minimum inhibitory concentration for the growth of *A. flavus* by direct addition was between 8,000 and 16,000 ppm, while for the vapor-exposure was between 4.7 and 9.4 ml of essential oil / l of air.

Significance: Although direct addition of EO has a rapid effect on *A. flavus* growth, exposure to EO vapors was more effective, requiring lower concentrations to achieve similar effect.

P3-101 Antibacterial Effectiveness of Phenyllactic Acid against Gram-positive and Gram-negative Foodborne Pathogens at pH 6.0 and 7.2

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Developing Scientist Competitor

Introduction: Consumers are increasingly demanding food products without synthetic food preservatives. In response to this demand, food manufacturers are exploring the use of food additives from natural sources as replacements for some of the traditional food preservatives.

Purpose: The present study was conducted to evaluate the antibacterial effectiveness of phenyllactic acid (PLA), produced by certain *Lactobacillus* species, against four foodborne pathogens.

Methods: Brain heart infusion broth (BHI) with added PLA at 0.0 (control) 0.312, 0.625, 1.25, 2.5, 3.75, 5.0, 7.5, 10 or 15 mg/ml, was inoculated with a multiple-strain cocktail of *Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus,* or *Escherichia coli* O157:H7 to give a final concentration of 6.0 log CFU/ml. Growth of the pathogens for 24 hours in BHI (pH 6.0 or 7.2) at 35 °C was monitored using a Bioscreen C turbidometer to measure optical density of the cultures at 600 nm. To determine whether PLA exerted a bacteriostatic or bactericidal effect, viable counts in broth with the respective minimum inhibitory concentration (MIC) of PLA for each pathogen were evaluated by surface-plating serially diluted (10-fold) broth samples on tryptic soy agar supplemented with 0.6% yeast extract and counting bacterial colonies after 24 hours of incubation (35 °C).

Results: Increasing levels of PLA extended the lag phase and decreased the growth rate of all four pathogens. The MICs of PLA at pH 6.0 and pH 7.2 were 2.5 and 5.0 mg/ml, respectively, irrespective of the target organism. PLA exhibited a bactericidal effect at its MIC for each pathogen with reductions in viable counts ranging from ~0.6 log CFU/ml (*E. coli* O157:H7) to 2.0 log CFU/ml (*S. aureus*) after 24 hours of exposure to PLA.

Significance: Based on these results PLA exhibits good potential as a natural antimicrobial for controlling growth of foodborne bacterial pathogens. Further research of the antimicrobial activity of phenyllactic in appropriate food systems is warranted.

P3-102 Use of Natural Preservation Solutions for Bakery Products

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Introduction: Shelf life of bread and other bakery products is determined by a number of factors, of which spoilage by molds can be considered one of the most important. Calcium-propionate is commonly added to bakery products to prevent mold outgrowth, however, consumers are becoming more hesitant and reluctant to consume products with chemical additives such as preservatives. By using a natural fermented sugar containing organic acid, no preservatives need to be claimed on the package, while the shelf life of the bakery products can be maintained.

Purpose: The objective is to evaluate the efficacy of natural fermented sugar products versus chemical Ca-propionate in preventing mold outgrowth in bread.

Methods: Baking trials using a standard recipe for white pan bread (100 g flour, 6 g compressed yeast, 0.5 g SSL, 58 g ice water, 1 g NFDM, 2 g salt, 8 g sugar and 2 g vegetable oil) were performed. The preservative action of adding 1.5% of liquid fermented sugar and 0.6% of fermented sugar powder was compared to 0.3% Ca-propionate. After baking, one bread was used to measure its product characteristics, such as pH and A_w, and a minimum of 4 breads were placed in closed, plastic bags and evaluated daily for visual mold spoilage. The first appearance of mold was used to define shelf life.

Results: The study shows that 1.5% of the liquid fermented sugar increased the time to first mold appearance with one day compared to 0.3% Ca-propionate. The powdered fermented sugar product did not give extra days of shelf life, but reached the same shelf life as the Ca-propionate control. The impact of the different preservatives on pH and A_w values was limited, with pH of 5.5 ± 0.1 and A_w of 0.948 ± 0.02 for all three preservatives tested.

Significance: This research shows a fermented sugar product providing at least equal shelf life for bakery products as chemical Ca-propionate, thereby providing the bakery industry with a natural solution for preservation.

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P3-103 Growth Comparison of *Listeria monocytogenes* in Laboratory Media Using Equimolar Concentrations of NaCl and KCl

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Introduction: In an effort to reduce sodium content in foods, manufacturers frequently replace sodium with potassium salts. However, due to differences in molecular weights, substituting salts on a percentage basis may result in reduced microbial inhibition.

Purpose: To compare the inhibition of *Listeria monocytogenes* (Lm) by various molar concentrations of NaCl and KCl.

Methods: Trypticase soy broth (includes 0.6% or 102 mM NaCl in the base) adjusted to pH 5.2 or 6.2 with HCl, was supplemented with an additional 0, 250, 500, 750 and 1,000 mM NaCl or KCl or equimolar mixture of NaCl: KCl to provide a total of 500 or 1,000 mM, and sterilized by autoclaving. Treatments were inoculated with 3-log CFU/ml Lm (3-strain mixture) and five tubes/ treatment assayed three times per week for changes in $absorbance_{600\ nm}$ during storage at 4 and 7 °C for 8 weeks. In addition, treatments were analyzed for %Cl-, pH, and a_w.

Results: When compared on a molar basis, there was no difference in a_w for NaCl vs. KCl even though the w/v concentrations of the KCl salts were greater than the NaCl treatments; a_w values were 0.99, 0.98, 0.975, 0.965, and 0.960 for the 0, 250, 500, 750 and 1,000 mM salts

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treatments, respectively. Similarly, time until growth ($A_{600\ nm}$ increase by 0.1) was not substantially different between the two salts. For example, at 4 °C the pH 6.2 Control grew at D-19, whereas growth was delayed until an average 22, 28, and 32 days for the 500, 750 and 1,000 mM salt treatments. Interestingly, Lm growth was slightly enhanced in the 250 mM added NaCl treatments compared to the Control regardless of the pH or storage temperature.

Significance: This study suggested that KCl will provide equivalent antilisterial activity as NaCl when substituted on a molar basis. Additional studies should confirm efficacy in foods and with other pathogens.

P3-104 Spiral Gradient Testing and the Mechanism of Resistance of Fluroroquinolone-resistant *Listeria monocytogenes* Isolated from Various Food Products

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Introduction: *Listeria monocytogenes* is widely distributed in the environment and is considered as leading causes of foodborne illnesses in the United States. Generally, *Listeria* is susceptible to multiple antibiotics, but resistant to selected quinolones. However, during the last several years, increasing numbers of strains isolated from various foods have been reported to resistant to multiple antimicrobial agents.

Purpose: The objective of this study was to determine the antimicrobial susceptibility of *Listeria* strains isolated from ready-to-eat foods and examine the presence of mutations in their gyrase A and B (*gyr*A and *gyr*B) genes.

Methods: We determined the Gradient Minimum Inhibitory Concentration (GMIC) and End Concentration (EC) of seven antimicrobial agents for 35 strains of *Listeria* isolated from various foods (chicken breast, soft cheeses and produce) using a spiral gradient endpoint method.

Results: Results of this experiment showed that 79%, 72%, 70% and 85%, 12%, 58% and 42% of the strains were resistant to ciprofloxacin (GMIC = 3.33 µg/ml; EC = 2.42 µg/ml) enrofloxacin (GMIC = 2.7 µg/ml; 1.74 µg/ml), norfloxacin (GMIC = 10.91 µg/ml; EC = 4.71 µg/ml) and ofloxacin (GMIC = 7.1 µg/ml; EC = 4.83 µg/ml), tetracycline (GMIC = 4.1 µg/ml; EC = 5.08 µg/ml), trimethoprim (GMIC = 61.61 µg/ml; EC = 31.14 µg/ml) and ampicillin (GMIC = 17.7 µg/ml; EC = 12.8 µg/ml), respectively. To determine the mechanism of quinolone resistance of *Listeria*, 10 food isolates were randomly selected and the sequences were analyzed. The analysis of quinolone resistance determining regions (QRDRs) of both the *gyr*A and B genes were determined. Amino acid substitutions were found in several strains for both *gyr*A and B gene. All ten strains exhibited distinct PFGE patterns when compared to each other.

Significance: Our study suggested that resistance to quinolone can be attributed to mutations in QRDRs of both the *gyr*A and B gene and this genetic mutation can be universally found in multiple strains of *L. monocytogenes* isolated from various foods.

P3-105 Characterization of Antimicrobial Resistance in *Vibrio parahaemolyticus* Isolated from Canada between 1998 and 2011

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Introduction: *Vibrio parahaemolyticus* is a naturally occurring marine organism with a worldwide distribution in estuaries and coastal environments. Illness associated with *V. parahaemolyticus* derives from contaminated seafood, with recent data showing increased rates of infection in British Columbia (BC). Although antimicrobial resistance (AMR) in *V. cholera* is well described, little data on *V. parahaemolyticus* AMR in Canada exists.

Purpose: Examine AMR and characterize clinical and environmental strains of *V. parahaemolyticus* isolated from Canada between 1998 and 2011.

Methods: In total, 360 *V. parahaemolyticus* strains, including clinical (n=64) and environmental (n=296) isolates, were selected for analysis. Environmental strains were predominantly isolated from BC, with 18 strains isolated from Atlantic Canada. AMR phenotypes were determined by disc diffusion assay using a panel of 20 antimicrobial agents. All strains were screened for the presence of toxin genes (*tdh*, *trh*) by multiplex PCR.

Results: Of the 360 *V. parahaemolyticus* isolates analyzed, 53% (n=34) and 7% (n=20) of the clinical and environmental strains, respectively, possessed *tdh* and/or *trh*. Overall, *V. parahaemolyticus* isolates were susceptible to most antibiotics. The most common resistances observed were to sulfisoxazole (95.3%), ampicillin (90.0%), cephalothin (28.3%), streptomycin (3.6%), and piperacillin (2.2%). Reduced susceptibility to various antimicrobials was observed, including erythromycin (99.7%), kanamycin (47.8%), polymyxin B (23.6%), ceftiofur (16.4%), ciprofloxacin (3.9%), trimethoprim/sulfamethoxazole (2.2%), enrofloxacin (1.1%), and oxolinic acid (1.1%). Resistance to three or more antibiotics was observed in two (3.1%) clinical and 96 (32.4%) environmental strains, whilst two pathogenic strains isolated from one BC geographic area were resistant to five antimicrobial agents.

Significance: AMR profiles of *V. parahaemolyticus* strains characterized in this study are similar to data published in other countries. Although overall levels of AMR are low, reduced susceptibility to extended-spectrum β-lactams and fluoroquinolones is noteworthy. Current work is being performed to further characterize these phenomena.

P3-106 Effect of Sodium Alginate Coatings Containing Cinnamon Essential Oil on Growth and Ochratoxin A Production by *Aspergillus carbonarius* on Fresh Apples and Pears

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Developing Scientist Competitor

Introduction: Mycotoxigenic fungi mainly contaminate fruits through damaged or wounded tissues and release toxin under improper storage conditions. Edible coatings containing natural antimicrobials can be applied on fruits to control fungal growth and toxin production.

Purpose: To evaluate the effect of sodium alginate coatings with or without cinnamon essential oil (EO) on Aspergillus carbonarius growth and OTA production on apples and pears.

Methods: Fresh apples and pears were intentionally wounded (0.5 cm) on the skin. Additional fruits were cut in slices (1 cm). Aliquots of A. carbonarius spore suspensions were added in the wounds (10^2 spores/g) or deposit on slices. Fruits were coated with sodium alginate (3% w/v) without (EF) or with EO of cinnamon at 0.3% (0.3 EFC) and 0.9% v/v (0.9 EFC) or left uncoated (C). Samples were stored at 15, 20 and 25 °C. Fungal growth was estimated by colony diameter measurements (n=20) and spores density (log spores/g) on MEA. Total viable counts (n=4) were enumerated on PCA. OTA production was determined by HPLC.

Results: EO was more effective in delaying growth of *A. carbonarius* at lower temperatures. 0.9% of cinnamon was more effective against fungus on slices than on skin, causing 100% inhibition of growth and OTA production on both fruits. However this treatment caused flesh browning. On

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pears, 0.3 EFC also prevented appearance of visible mycelium and OTA production. EF did not significantly influence *A. carbonarius* growth on skin and slices. However, slices with EF showed ca 3-fold higher OTA compared to controls at 20 and 25 °C. At 15 °C, OTA production followed the order C> EF> 0.3 EFC> 0.9 EFC. Higher amounts of OTA were observed on slices than on skin; for instance, at 20 °C average OTA amounts of 3 ppm was produced on pears slices, while 1.5 ppm on skin. Fungal growth was more extensive and OTA concentration was higher on pears compared to apples.

Significance: Edible coatings containing cinnamon may assist to control fungal growth and OTA production on fresh fruits.

P3-107 Antimicrobial Synergistic Effect of Selected Essential Oils/Derivatives against *Salmonella* Typhimurium

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Introduction: Antimicrobial effect of essential oils and their application as food additives to enhance aroma, flavor, and safety of food is well-known. However, there are limited studies on the synergistic effect of these natural antimicrobial agents on microbial growth.

Purpose: In the present study, the inhibitory effect of six essential oils/derivatives (basil, thyme, nutmeg and oregano oil, trans-cinnamaldehyde, and eugenol), their binary mixtures, and emulsions was investigated on *Salmonella* Typhimurium ATCC 19585 in a broth model system.

Methods: Minimum Inhibitory Concentration (MIC) and diameters of Zone of Inhibition (ZOI) of oils/derivatives dissolved in ethanol against *S.* Typhimurium were determined utilizing Broth Dilution Method and Disc Diffusion Method, respectively. Synergistic effect was monitored by Checkerboard assay method and then Fractional Inhibitory Concentration (FIC) was calculated. Oil-in-water emulsions were prepared by sonication utilizing Tween 80 as an emulsifier. MICs were then evaluated on *S.* Typhimurium.

Results: Trans-cinnamaldehyde and oregano oil were most effective in *S.* Typhimurium inhibition (ZOI: 22, 21 mm; MIC: 312.5 ppm), followed by eugenol and thyme oil (ZOI: 19, 14 mm; MIC: 625 ppm), and nutmeg oil and basil oil (ZOI: 14, 15 mm; MIC: 1250 ppm). All binary combinations of oils exhibited synergistic effect as indicated by the FIC ≤ 0.5, though MIC values were higher for emulsions (nutmeg and basil oil: >5000 ppm; eugenol: 5000 ppm; thyme oil: 2500 ppm; oregano oil, trans-cinnamaldehyde: 625 ppm) than those for the oils dissolved in ethanol.

Significance: Combinations of essential oils/derivatives have potential to be used as food additives for increased safety.

P3-108 A Longitudinal Study of Antimicrobial Resistance of *Vibrio parahaemolyticus* Strains Isolated from Two Statistical Areas in British Columbia, Canada

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Introduction: *Vibrio parahaemolyticus* is a naturally occurring marine organism with a worldwide distribution in estuaries and coastal environments. Longitudinal data examining the dynamics of antimicrobial resistance (AMR) patterns of *V. parahaemolyticus* in Canada is limited.

Purpose: Examine AMR and characterize environmental strains of *V. parahaemolyticus* isolated from two British Columbia geographic areas between 1998 and 2011.

Methods: In total, 85 *V. parahaemolyticus* strains isolated from the Department of Fisheries and Oceans Statistical Area (SA) 13-15 (n=24) and SA 14-08 (n=61) were selected for analysis. AMR phenotypes were determined by disc diffusion assay using a panel of 20 antimicrobial agents. All strains were screened for the presence of toxin genes (*tdh*, *trh*) by multiplex PCR.

Results: Of the 85 *V. parahaemolyticus* isolates analyzed, two (2%) possessed *tdh* and/or *trh*. The prevalence of resistance to specific antimicrobials between 2007 and 2011 at SA 13-15 were: ampicillin, 92%; sulfisoxazole, 83%; cephalothin, 17%; and streptomycin, 8%. Compared to isolates collected between 1998 and 2001 (n=12), resistance to streptomycin increased while a decrease was seen for cephalothin and sulfisoxazole. The prevalence of AMR among isolates originating from SA 14-08 between 2008 and 2011 (n=38) are as follows: ampicillin, 100%; sulfisoxazole, 89%; cephalothin, 29%; piperacillin, 5%; streptomycin, 5%; and gentamicin, 3%. Compared to isolates collected between 1998 and 2000 (n=23), increased resistance to ampicillin, piperacillin, streptomycin, and gentamicin were noted while a decrease was observed for cephalothin and sulfisoxazole.

Significance: Although overall levels of AMR were low, isolates resistant to four or more antibiotics were only observed between 2008 and 2011. Reasons for this, however, are not currently understood and further study is required to gain additional insight.

P3-109 Reduction of *Salmonella* Using Lactic Acid and Potassium Lactate on Non-federally Inspected Whole Muscle Beef Steaks Purchased in Meat Markets of Mexico

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Introduction: In Mexico, foodborne illnesses are the leading cause of death in small children. These cases occur because of contaminated products that are not properly handled. Recent data indicate that product in the "wet markets" is commonly contaminated with *Salmonella* and produced in plants that are not federally inspected.

Purpose: To determine the effectiveness of a 5% lactic acid dip and a 5% potassium lactate dip to reduce *Salmonella* on naturally contaminated beef purchased in wet markets in Mexico.

Methods: Non-federally inspected beef whole muscle steaks (approximately 100 grams each) were purchased from city markets, street vendors and butcher shops across 4 cities in Mexico. In the first experiment, samples (n=362, 2 replications) were dipped in 5% lactic acid solution. The second study, samples (n=722, 4 replications) were dipped in a 5% potassium lactate solution. In both studies, duplicate non-treated steaks serve as controls. For each sample, the presence of*Salmonella* was determined with the BAX® system, and if confirmed, MPN enumeration was performed and colonies confirmed using a latex test. All data was analyzed with Statistical Analysis System (SAS 9.3) software.

Results: Lactic acid study controls, resulted in 44.48% positive for *Salmonella* as compared to 34.25% positve after the lactic acid dip. Lactic acid treatment revealed an average *Salmonella* level of 2.21 MPN/g log /50cm², significantly less than the controls, 2.80 log /50cm² ($P < .0001$).

Potassium lactate treatment resulted in 31.72% positive for *Salmonella* compared to controls (34.49% positive). There was a statistically significant difference in the controls versus treated samples counts which was 3.15 and 2.81 MPN/g log /50cm², respectively ($P < 0.0073$).

Significance: Results indicated that in Mexico there is an unacceptable amount of beef contaminated with *Salmonella,* but the lactic acid and potassium lactate dips can reduce its levels, providing safer beef products to the citizens of Mexico.

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P3-110 Antibacterial Effect of ZnO Nanoparticles on Intestinal Bacteria

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Introduction: The application of engineered nanoparticles (NPs) for food safety is increasingly being explored. Zinc oxide (ZnO) NPs are inorganic chemicals with exceptional biocompatibility, non-toxicity and bioactivity characteristics and considered safe for use in the pharmaceutical and medical industries. However, not much is known about the behavior of such NPs upon ingestion and whether they inhibit natural gut microflora.

Purpose: The objective of this study was to investigate the effects of ZnO on the intestinal bacteria *Escherichia coli*, *Lactobacillus acidophilus* and *Bifidobacterium animalis*.

Methods: ZnO NPs at concentrations of 0, 12, 16, and 20 mM were used in tube dilution tests. Tryptic soy broth or Lactobacilli MRS broth without NPs and containing NP-free solution were used as controls. Cells were exposed to the different ZnO NPs concentrations in broth for 24 h, before plating on respective agar medium for each bacterial strain.

Results: At the end of 24 h of exposure, numbers of all three bacterial strains did not significantly differ from those of the controls. Growth of *L. acidophilus* and *B. animalis* initially slowed up to 10 h, but their numbers increased to within 1 log CFU/ml or less compared to the control by the end of 24 h. No significant differences were found in cell reduction among the different concentrations of ZnO NP tested.

Significance: This study revealed that ZnO NPs had no significant inhibitory effect on *E. coli*, *L. acidophilus* and *B. animalis*. Further research is ongoing to examine the effect of other engineered NPs on intestinal bacteria and intestinal epithelial cells.

P3-111 Influence of Nalidixic Acid Resistance on Sensitivity of Various Shiga Toxin-producing *Escherichia coli* to EO Water Treatment

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Introduction: Intervention studies on inactivation of Shiga toxin-producing *E. coli* (STEC) from beef are often complicated by high level of background microflora. One solution to this problem is to use antibiotic-resistant pathogen strains.

Purpose: This study was designed to validate the use of nalidixic acid-resistant (NalR) strains of various STEC for electrolyzed oxidizing (EO) water efficacy study.

Methods: The resistance of total sixty-two strains (parent strains {NalS} and NalR) of *E. coli* O157:H7 and six major serotypes of non-O157 STEC was tested against EO water using minimum inhibitory concentration (MIC) and inoculated beef trims. MIC was conducted for 15 s testing period with free chlorine concentration of 3.00, 2.50, 2.00, 1.50, 1.00, 0.50 and 0.25 mg/l. Beef pieces (5 cm cube, 25 -35% fat) were inoculated with same serogroup strains cocktail and treated with EO water (70mg/l available chlorine, pH 2.5, ORP 1175mV) for 5 min. At the end of each treatment period, beef pieces were promptly placed in 2X neutralizing buffer and bacterial counts were determined by plating appropriate dilutions on plate count agar and MacConkey agar plates (supplemented with or without 50 mg/l nalidixic acid).

Results: The MIC of individual strains ranged from 0.25 to 1.50 mg/l free chlorine of EO water. Resistance pattern of cocktails was determined as *E. coli* O157 ≥ O103 ≥ O26 ≥ 0111 ≥ O121 ≥ 045 > O145. When treated on beef trim similar pattern of resistance was observed with pathogen load reduction ranging from 1.5 to 2.8 CFU/cm^2. No significant differences in sensitivity towards EO water treatment were observed between NalS and their NalR derivatives in either method.

Significance: No significant difference in resistance of NalR and NalS were found which validates the use of NalR strains in EO water efficacy study. In addition, the EO water treatment that reduces *E. coli* O157:H7 could equally or more effectively reduce non-O157 STEC from beef trim.

P3-112 Concentration- and Time-dependent Inactivation of *Escherichia coli* O157:H7 by Plant Extracts

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Developing Scientist Competitor

Introduction: Virulent foodborne *Escherichia coli* O157:H7 are reported to cause dangerous enteric disease in humans. The emergence of *E. coli* O157:H7 strains that resist inactivation by medicinal antibiotics may further enhance the danger of these pathogens to animals and humans. In an effort to find alternate natural novel compounds that can prevent the growth of *E. coli* O157:H7, we investigated the antimicrobial activities of plant extracts.

Purpose: The objective was to compare the antimicrobial activities of the following plant extracts against *E. coli* O157:H7 in laboratory media: apple polyphenol; black, decaffeinated black and green tea extracts; and grapeseed extract.

Methods: Four concentrations of dehydrated powders (4%, 3%, 2% and 1%) were each dissolved or suspended in sterile phosphate-buffered saline solution. These solutions/suspensions were then added to the test bacterial cultures ($10^{3\text{-}4}$CFU/µl), mixed thoroughly, and incubated at 37 °C for various time periods. Aliquots taken at five time periods (0, 1, 3, 5 and 24 hours) were then enumerated for surviving bacterial populations.

Results: All plant extracts exhibited strong concentration- and time-dependent *in vitro* antimicrobial activity against *E. coli* O157:H7. Green tea at ≥ 2% reduced the bacterial population below detection (3-4 logs) after 1 h, and after 3 hours at a concentration of 1%. Grapeseed extract (1-4%) at 3 h induced reduction of the pathogen below detection limits. Apple polyphenol at 1-2% reduced pathogen population to below detection levels at 3 h; at 4% induced 2.5-log reduction at 1 hr; and at 2-3 % reduced bacterial population to below detection at 5 h. Black tea at ≤ 2% produced ~3 log reduction in 3 h. Decaffeinated black tea induced a 2-log reduction at 3 h and complete reduction at 24 h.

Significance: This research provides potential antimicrobial candidates against *E. coli* O157:H7 in various milieus, including human food and animal feed formulations.

P3-113 Isolation and Characterization of a Unique Phage Carrying Strain of *Clostridium botulinum* from Carrot Juice

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Introduction: *Clostridium botulinum* is an important foodborne pathogen capable of forming thermally resistant endospores and producing deadly botulinum neurotoxins (BoNTs). In 2006, this pathogen was responsible for an international outbreak of botulism attributed to the consumption of commercially prepared carrot juice, resulting in six cases including one death. From this outbreak, the CDC isolated two genetically distinct strains of *C. botulinum* namely, CDC51303 and CDC51348 from incriminated carrot juice bottles.

Purpose: The purpose of this study was to isolate additional strains of *C. botulinum* that may have contributed to the high quantities of BoNT detected in the adulterated product.

Methods: Additional carrot juice bottles retrieved from the facility during the outbreak were used to plate out and obtain toxin-producing isolates which were subsequently analyzed using pulsed-field gel electrophoresis (PFGE) and Southern hybridization analysis.

Results: None of these additional toxigenic isolates tested exhibited a restriction banding pattern similar to strain CDC51348. PFGE of *Xho*I and *Sma*I digested DNA samples showed that isolates CJ4-1 and CJ10-1 shared an identical pulsotype to strain CDC51303. Although CJ5-1 exhibited an identical PFGE pattern to CDC51303 when *Sma*I was used as the restriction enzyme, this strain displayed a unique pulsotype when PFGE was performed using DNA digested with *Xho*I. CJ4-1 and CJ5-1 were selected for further analysis using a focused DNA microarray. This analysis revealed several phage related genes present in CJ5-1, but which were absent in CJ4-1. Southern hybridization analysis of digested and non-digested DNA of CJ4-1 and CJ5-1 using probes specific for each phage gene suggested their chromosomal rather than extrachomosomal location.

Significance: The acquisition or loss of bacteriophages has been demonstrated among strains of *C. botulinum* in a food, clinical or environmental sample. This phenomenon presents a challenge for the timely, accurate identification of an outbreak strain during a botulism outbreak investigation.

P3-114 Expression of Stress and Virulence Genes in *Escherichia coli* O157:H7 in Fresh Dairy Compost

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Introduction: Composting process has been used to inactivate foodborne pathogens in animal wastes on farm. However, the extended survival of these pathogens during composting has been reported. Heat-adaptation of pathogen during the mesophilic phase of the composting process may induce stress response, thereby extending their survival.

Purpose: This study was to understand the mechanisms for pathogen survival during composting by analyzing expression patterns of selected stress and virulence genes.

Methods: A two-step real-time PCR assay was used to evaluate expression of stress and virulent genes in *Escherichia coli* O157:H7 heat-shocked in compost. *E. coli* O157:H7 (strain F07-020-1) was inoculated in autoclaved fresh dairy compost which was heat-shocked at 47.5 °C for 10 min in water bath. To serve as medium control, heat-shock of the pathogen was also conducted in tryptic soy broth (TSB).

Results: In compost, heat-shock genes (*clpB, dnaK, groEL*) and alternative sigma factor (*rpoH*) of *E. coli* O157:H7 were all up-regulated significantly ($P < 0.05$). There was no significant ($P > 0.05$) difference in the expression of trehalose synthesis genes. Virulent genes such as *stx1* and *fliC* were up-regulated while the rest of genes were down-regulated with no significant difference ($P > 0.05$). In toxin-antitoxin system, toxin genes, *mazF*, *hipA*, and *yafQ*, were up-regulated with no significant ($P > 0.05$) difference, whereas antitoxin gene *dinJ* was up-regulated with level of expression significantly ($P < 0.05$) different. Most of other antitoxin genes were down-regulated. In broth as the heat-shock medium, all heat-shock genes were up-regulated with relative fold change significantly ($P < 0.05$) different. There was no significant ($P > 0.05$) change in trehalose synthesis genes in broth medium either. Except for *eaeA*, the rest of virulent genes were down-regulated with no significant ($P > 0.05$) change. Majority of the toxin-antitoxin genes were down-regulated with relative fold change in toxin genes *hipA* and *chpB* only significantly ($P < 0.05$) different.

Significance: Our results suggest that induction of heat-shock response in pathogens plays an important role in providing protection to pathogens against lethal temperature during composting. Activation of toxin-antitoxin (TA) system in addition to heat-shock response may also be supporting pathogen survival in compost as a parallel mechanism.

P3-115 *Enterococcus faecium* NRRL-B2354 as a Surrogate for *Salmonella* spp. for the Validation of Extrusion

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Introduction: Multiple outbreaks of salmonellosis have been associated with the consumption of low-moisture products including extruded products such as breakfast cereals and pet foods. Although these outbreaks are uncommon, they generally affect large numbers of consumers due to the widespread use of these products. Therefore, there is a need for a non-pathogenic, surrogate microorganism that can be used to validate extrusion processes for *Salmonella* spp.

Purpose: The objective of this research was to determine if *Enterococcus faecium* NRRL B-2354 is an adequate surrogate organism for *Salmonella* spp during extrusion by comparing the temperature profiles at which each organism is inactivated.

Methods: A balanced carbohydrate-protein mix was formulated to 28% moisture and inoculated with pure cultures of either *Enterococcus faecium* NRRL-B2354 or a five-strain cocktail of heat resistant *Salmonella enterica* to a final level of 5 logs per gram or above. In each experiment, the mix was processed in a pilot scale extruder using temperatures from 60 to 110 °C in 5 °C intervals. At each evaluated temperature, the extruder was allowed to equilibrate for 10 minutes before sample collection. Samples were collected in sterile bags, cooled in dry ice, and stored at 4 °C prior to analysis. These were then enumerated for *Salmonella* using Tryptic Soy Agar (TSA) with an XLT4 overlay and also in XLT4 alone, followed by incubation at 37 °C for 24 h. *E. faecium* was enumerated using both TSA and mEnterococcus media followed by incubation at 35 °C for 48 hours. The extrusion process for each organism was replicated twice and from each extrusion, three subsamples were evaluated for microbial counts. Survival curves were then plotted based on enumeration data (log CFU/g) and temperature to compare the heat resistance of each organism.

Results: According to the data collected, the minimum temperature needed to achieve a 5 log reduction of *E. faecium* was 73.5 °C in this food matrix. Above 80 °C, *E. faecium* steadily decreased until undetectable levels (<10 CFU per g) were reached ($R^2 = 0.813$). *Salmonella* spp were reduced by 5 logs at 61.0 °C, and above 68.0 °C they decreased until undetectable ($R^2 = 0.777$).

Significance: The data shows that *E. faecium* NRRL-B-2354 is inactivated at a higher temperature than *Salmonella*, indicating that its use as a surrogate would provide an appropriate margin of error in extrusion processes designed to eliminate this pathogen. With increased regulatory pressure to minimize risk, the industry will be required to validate different formulations in combination with varying thermal treatments, and the use of *E. faecium* presents itself as a safer alternative for those validation studies.

P3-116 Colonization and Internalization of *Salmonella enterica* in Tomato Plants

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Introduction: The consumption of fresh tomatoes has been linked to numerous foodborne outbreaks involving various serovars of *Salmonella enterica*. Recent advances in our understanding of microbial-plant interactions have shown that human enteric pathogenic bacteria,

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including *S. enterica*, are adapted to survive in the plant environment.

Purpose: In this study, tomato plants were inoculated with *S. enterica* serotypes to evaluate plausible internalization routes and to determine if there is any niche fitness for certain serovars.

Methods: A cocktail of five *S. enterica* serotypes was used to inoculate tomato plants (cultivar: Micro-Tom) by spotting leaflets or brushing blossoms with inoculum, or by inoculating soil right after transplanting. Molecular serotyping was used to survey serotypes of 100 colonies, each randomly picked from *Salmonella* positive soil-root, leaflet, and blossom samples. Stem walking was used to trace the internal movement of *Salmonella* from the root. Both surface and interior of green or ripe tomato fruits were examined for *Salmonella* as well.

Results: Soil inoculation led to *Salmonella* internalization in 3 of 40 plants, and contamination of one of 30 tomatoes assayed. Of 71 tomatoes harvested from inoculated blossoms, 49 (69%) surface samples were positive for *Salmonella*, and 22 (31%) interior samples were positive from the 49 *Salmonella* positive tomatoes. Of the five serotypes inoculated, Newport and Javiana were the most dominant in soil at both days 8 and 23. On tomato leaflets, although all five serotypes, except Typhimurium, were evenly distributed at day 8, only Newport and Montevideo remained dominant at day 23. Montevideo was the most prevalent serotype on blossoms at day 7.

Significance: Results suggest that soil and tomato blossoms are possible sites for *Salmonella* internalization, and for the first time, *Salmonella* serotype-specific niche adaptation on the tomato plant was demonstrated.

P3-117 Influence of Lipoteichoic Acid (LTA) on *Listeria monocytogenes* Biofilm Formation

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Introduction: Lipoteichoic acid (LTA) is an important polymer on the surface of most Gram-positive bacteria. In *Listeria monocytogenes* (*L. monocytogenes*) transposon mutagenesis identified *lmo2555* to be involved in the synthesis of the glycolipid backbone that links the LTA to the cell membrane and phenotypically this gene appears to be critical for initial adhesion and biofilm formation.

Purpose: The purpose of this study is to synthesize a deletion mutant of *Lmo2555* and further characterize the functionality of LTA in *L. monocytogenes* biofilm formation.

Methods: Deletion mutant of *Lmo2555* (Δlmo2555) was created by PCR amplification using two primer pairs, each with restriction sites upstream and downstream of the targeted gene. The two parts were joined together creating a deleted version of *Lmo2555*, which was cloned into a temperature sensitive shuttle vector pKSV7 in *E. coli.* The recombinant plasmids were transformed into *L. monocytogenes* wild type strain (LM21). Allelic exchange was performed in a two-step process. Initially, transformants were maintained under antibiotic selection at an elevated temperature (41 °C) which allows selection of cells which had undergone homologous recombination. Integration was confirmed by PCR amplification with primers specific for downstream region of pKSV7 plasmid and an upstream region of the chromosome. Selection for colonies that had undergone allelic exchange was performed by incubating isolates without antibiotic selection at a plasmid replication permissive temperature (32 °C).

Results: Approximately 360 colonies were screened for plasmid loss by antibiotic sensitivity and allelic exchange was confirmed using PCR amplification. Deletion mutant (Δlmo2555) showed a significantly less biofilm formation than wild type strain (LM21), indicating that LTA is involved in biofilm formation and in the structure of *L. monocytogenes.*

Significance: An understanding of the biological influences on biofilm formation in *L. monocytogenes* will ultimately result in unique strategies to prevent and remove biofilms in food processing environment.

P3-118 Frequency of Bacterial Foodborne Pathogens on the Surface and Guts of Individual Filth Flies

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Introduction: It is recognized that some species of flies are more likely to transmit foodborne pathogens. However, more data is required to properly assess the risk associated with the presence of a particular fly species in food or food facilities and the ability of an individual fly to vector foodborne pathogens.

Purpose: To establish the frequency of *Salmonella* spp., *Cronobacter sakazakii* and *Listeria* spp. from the surface and guts of individual flies collected from dumpsters outside restaurants in urban areas.

Methods: A total of 100 flies were collected and identified from 10 randomly selected sites. Pathogen detection on the body surface and guts of the flies was performed using the BAX® System Q7 (DuPont Qualicon) followed by confirmation of BAX positive samples using the BAM protocol described for each bacterial pathogen. Presumptive *Salmonella* spp. and *Listeria* were confirmed through ribotyping, and presumptive *C. sakazakii* were confirmed using chromogenic media and PCR amplification and sequencing of 16S rRNA and α-glucosidase genes.

Results: The most abundant fly species was *Musca domestica* (47%), followed by *Lucilia cuprina* (33%), *Lucilia sericata* (14%) and others (6%). On the body surface of individual flies, *C. sakazakii* (7%), *L. monocytogenes* (1%) and *Salmonella*serovar Poona (1%) were isolated and confirmed. Whereas *C. sakazakii* (16%), *L. monocytogenes* (3%), *L. innocua* (4%) and *Salmonella* serovars Poona (1%), Hadar (1%), Schwarzengrund (1%), Senftenberg (1%) and Brackenridge (1%) were isolated and confirmed in the guts of collected flies.

Significance: This study provides data in support of insect control to minimize the spread of bacterial pathogens carried by an individual filth fly species. *L. monocytogenes* and all but one isolated *Salmonella* serovar have been implicated in food poisoning outbreaks reported by the CDC, thus emphasizing the importance of regulating disease-causing flies in food facilities to decrease the risk of foodborne illness.

P3-119 Assessing the Formation and Removal of Biofilms of *Listeria* spp. Isolated on Equipment and Utensils of Dairy Industries in Brazil and Italy

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Introduction: Currently, *Listeria* (*L.*) *monocytogenes* is one of the most important foodborne pathogens worldwide because it is an environmental psychrotrophic microorganism with very low infectious dose, able to cause high mortality rates.In addition, *Listeria* can form biofilms on surfaces that come in contact with food, resulting in food contamination.

Purpose: This study aimed to investigate the presence of *Listeria* spp. on equipment and utensils of dairy industries, and to investigate the formation and removal of biofilms formed by isolated microorganisms.

Methods: A total of 106 surface samples (100 cm^2) were collected in three dairy industries, two located in southern Brazil and one in southern Italy, using the 3M™ Swab-Sampler. Microorganisms were identified by ISO methods, BAX System (DuPont®) and Kit API *Listeria*®

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(BioMérieux). Isolated microorganisms were placed on AISI 316 stainless steel and polyethylene coupons, in order to evaluate the formation and removal of biofilms by different disinfectants. Statistical analyses were carried out using ANOVA, Tukey Test and t-Test.

Results: Seven samples were positive for *Listeria* spp., being 6 *L. innocua* and 1 *L. monocytogenes*. A bacterial pool formed by isolated strains was able to form biofilms of 4.34, 4.33, 4.40 and 4.43 log CFU/cm^2 on stainless steel coupons, andof 4.44, 4.40, 4.60 and 5.31 log CFU/cm^2 on polyethylene coupons, in time intervals of 15, 45, 90 and 180 minutes, respectively. The sanitizers 1% Peracetic Acid, 1% Sodium Hypochlorite and 2% Alkyl Dimethyl Benzyl Ammonium Chloride were able to completely inactive the biofilms after 1 and 10 minutes of exposure, demonstrating to be effective against *Listeria* tested.

Significance: *Listeria* spp. isolated from dairy industries are able to form biofilms on equipment and utensils, however proper use of sanitizers can eliminate these microorganisms.

P3-120 Comparative Survival Patterns of Non-O157:H7 Shiga Toxin-producing *Escherichia coli* (STEC) Strains and Acid-resistant O157:H7 STEC during Incubation in pH 2.0 Synthetic Gastric Fluid

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Introduction: Shiga toxin-producing *Escherichia coli* strains will encounter low pH during their passage through the human stomach before colonization in the intestine and resulting illness. The risk to human health presented by STEC strains therefore depends on their ability to survive in a low pH environment.

Purpose: This study compared the survival patterns of known acid-resistant O157:H7 strains (5 strains, including O157:H7 ATCC 43895), to those of acid-adapted but not acid-resistant non-O157:H7 STEC (3 strains each of serogroups O26, O45, O103, O111, O121, O145) in a model stomach system (synthetic gastric fluid).

Methods: Each strain was grown in Brain Heart Infusion broth (BHIB) for 24h to induce acid adaptation. Inocula were prepared by making serial dilutions in BHIB (to ca. 10^4 CFU/ml). Each strain (2ml) was combined with 18ml of pre-warmed SGF (pH 2.0; 37 °C) and incubated (shaken @ 100 rpm) at 37 °C for 6h. Sampling was conducted at 0h, 0.5h, 1h, 2h, 3h, 4h and 6h and cell counts were determined on modified Eosin Methylene Blue agar (MEMB). For each trial (n=3) of each strain, an exponential curve with detection limit was modeled to fit the data and generate two survival parameters: initial population (log CFU/ml) and decay rate (log CFU/ml/h).

Results: Decay rates ranged from 0.085 log CFU/ml/h to 10.11 log CFU/ml/h. Analysis of variance showed significant differences ($P < 0.05$) in initial log CFU/ml and decay rate, with significant differences between serogroups O103 and O157; O111 and O121; O121 and O45, O26, O145, O157; and O157 and O45. Significant differences ($P < 0.05$) were found in decay rates between the five O157:H7 strains. Overall, the lowest decay rate was exhibited by one O111 strain; one O121 strain had the highest decay rate.

Significance: These results suggest that non-O157:H7 STEC may survive well in the low pH environment of the human stomach, with the potential to produce illness. Further research is needed to investigate the survival patterns of non-O157 STEC in food products under similar conditions.

P3-121 Regulation of the csgD Promoter by Global Regulators H-NS, IHF, and RpoS in *Escherichia coli* O157:H7 Isolates

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Introduction: Curli production is essential for the biofilm formation in *E. coli* and *Salmonella*. The control of expression of CsgD, the key regulator of the curli operon, is very complex, and has been shown to be regulated by several global regulators and is influenced by various growth and stress conditions such as stationary phase, nutrient deprivation, temperature, and osmotic perturbation.

Purpose: We sought to understand the interplay between various global regulators and growth phase on the regulation of *csgD* promoter, and to find the promoter region that is required for *csgD* expression in the *E. coli* STEC strains.

Methods: Various lengths of the promoter region between *csgD* and *csgB* from a wild-type *E. coli* O157:H7 EDL933 strain (ATCC43895) and a strong curli-producing isolate were used to generate the *lacZ* translational fusions on a plasmid. The plasmids were then introduced into different strains carrying deletion of the potential regulators of *csgD*, such as *rpoS*, *himA*, and *hns*. β-galactosidase assays were performed throughout growth phases and the expression patterns were compared.

Results: We identified the minimal promoter length required for efficient *csgD* expression, as well as different regions that interact with the global regulators. Particularly, we found a ca. 600-bp *csgD* promoter was strongly affected in various mutants. The expression of this promoter was dramatically increased in all growth stages in the *rpoS* mutant compared to those of the wild-type. On the other hand, the *hns* and *himA* deletions only affected the expression of this *csgD*promoter in the late log to stationary phase.

Significance: By understanding the expression control of *csgD* gene, we gain insight into the regulation of curli production and biofilm formation. These *csgD* promoter fusions can be used as tools for evaluation of the biofilm-forming potential of the STEC isolates.

P3-122 Fate of *Salmonella* Exposed to Dry Heat Treatments in Low and Intermediate Moisture Food Products

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Introduction: *Salmonella*, a widespread vegetative pathogen of great concern to the food industry, has proven to be very heat sensitive under fully moist conditions. However, as the moisture content is reduced, the resistance to heat increases dramatically. Additionally, the survival rate of *Salmonella* under dry heat conditions has been found to vary significantly from product to product.

Purpose: The purpose of this study was to assess the lethality of *Salmonella* spp. in low to intermediate moisture foods employing dry heat. Currently, very limited information is publically available regarding the lethality in such products.

Methods: Nine strains of *Salmonella* were grown on TSA, harvested, and inoculated into soy protein and wheat flour products. The moisture levels of the products utilized were 5-8%, 12-15%, and 20-25%. Five-gram portions of product were placed in aluminum cups and baked for various time in a Fisher Isotemp 351 convection oven at temperatures ranging from 200 °F to 325 °F. *Salmonella* levels on untreated inoculated controls and baked samples were established by plating triplicate samples in duplicate on TSA overlayed with XLD agar. Survivor curves were plotted and D-values calculated and compared.

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Results: Log reductions for each product and treatment were calculated. Log reductions found varied significantly based on product type, moisture level, temperature and time of heat exposure. Low moisture (5%-8%) products processed at temperatures ranging from 200° to 325 °F varied considerably requiring 8 minutes up to 2,400 minutes of dry heat treatment to attain a 5+ log reduction whereas the intermediate moisture (20-25%) products showed less variability and were able to achieve a similar reduction in 180 seconds to 18.5 minutes.

Significance: These results show that the degree of variability in log reduction of *Salmonella* spp. under dry heat treatments decreases significantly as the moisture level increases. However, factors inherent to the product type (e.g., fat levels) can also impart variability.

P3-123 A Comparison of *Escherichia coli* Persistence on Basil Plants and Soil Using Drip and Overhead Irrigation

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Developing Scientist Competitor

Introduction: It is estimated that each year in the US there are 63,153 cases of foodborne illnesses caused by *E.coli* O157 serotypes and 112,752 illnesses caused by non-O157 Shiga toxin-producing *E.coli*. Irrigation water is recognized as a pre-harvest contamination source and has been linked with outbreaks of *E. coli* O157:H7 on leafy greens.

Purpose: In this study, the persistence of *E. coli* O104:H4, *E. coli* O157:H7 4407, and a cocktail of avian pathogenic *E. coli* O157 serotypes (APEC) on basil plants and soil using drip and overhead irrigation models was investigated.

Methods: Overnight cultures were individually inoculated into an irrigation solution containing a dairy and poultry manure slurry. Soil was inoculated with 3 ml of irrigation solution by pipette to model drip irrigation. Plants were sprayed with irrigation solution using an airbrush for 20 s (3 ml) to model overhead irrigation and resulted in 5.3, 5.8, and 5.3 log CFU/plant of *E.coli* O157:H7, *E.coli* O104:H4, and APEC on day 0, respectively. Plant and soil samples were taken on days 0 through 4 and bacterial populations were enumerated on selective media.

Results: *E. coli* were not detected on whole plants inoculated by drip irrigation. When overhead-inoculated by spray, bacteria persisted on plants at significantly higher populations ($P = 0.03$; $\alpha < 0.05$) with 2.3, 3.8, and 4.8 log CFU/plant of *E.coli* O157:H7, *E.coli* O104:H4, and APEC detected on plants 4 d post-inoculation, compared to persistence on drip-irrigated plants. Environmental APEC strains were recovered at significantly higher populations than outbreak strains on plants.

Significance: *E. coli* O157 serotypes and *E. coli* O104 did not persist on plants as readily as they did in soil. Environmental APEC persisted at higher populations than outbreak strains on basil plants. This study reinforces the notion that drip irrigation reduces the risk of contamination on foliar surfaces.

P3-124 Differentiating Non-0157:H7 STEC Serogroups from Ground Beef Plated on Agar Media by Hyperspectral Imaging

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Introduction: The development of an assay to detect and confirm a positive non-O157:H7 isolate is challenging when mixed morphologically results are obtained from the serogroups growing on Rainbow agar. Rainbow agar is only claimed by the manufacturer to be very specific for *E. coli* O157:H7 strains which produce black colonies. It is a bonus that non-O157 serogroups also produce colonies of various colors. Non-O157 serogroups can produce pink, purple, blue-purple, gray, gray-blue or gray-purple colonies which can be indistinguishable from each other and other background flora. The challenge for a microbiologist is selecting well isolated non-O157 STEC colonies from enriched samples plated on Rainbow agar for further testing and confirmation.

Purpose: To investigated the ability of hyperspectral imaging in differentiating non-O157:H7 STEC serogroups (O26, O45, O103, O111, O121, and O145) from ground beef background flora, serial dilutions of enriched ground beef samples spiked with STECs were spread onto agar plates for imaging.

Methods: Ground beef (65+2 g) was enriched in 585 +15 ml of modified tryptic soy broth (m-TSB, 20 mg/ml novobiocin) over night at 42 °C. Four ten-fold dilutions of the enriched ground beef sample were prepared in sterile saline. Then for each serogroup, approximate 1,000 CFU (10 ml of a 10^5 CFU/ml cell suspension) of STEC was spiked into 990 ml of each of the four enriched ground beef sample serial dilutions. The STEC spiked ground beef sample dilutions were thoroughly mixed then 50 and 100 ml of each dilution was spread onto individual Rainbow agar plates. The Themis Vision Systems' hyperspectral imaging system was used to acquire images from 400 nm to 900 nm. Regions of interest associated with non-O157 STEC colonies and background flora were created for validation using a previously developed Mahalanobis distance classifier.

Results: PCA score plots revealed potential separability serogroups from each other and the background flora. The prediction with six PCA components showed an overall detection accuracy of 94%. Detection accuracy varied from 88% to 100%. Sensitivity and specificity of hyperspectral imaging in detecting the target organism and differentiating the target from the background flora was 93% and 100%, repsectivley.

Significance: The potential of combining hyperspectral imaging and chemometrics to differentiate non-O157 STEC serotypes from background flora was demonstrated. Hyperspectral imaging can improve the speed and accuracy of selecting well isolated non-O157 STEC colonies from enriched samples plated on Rainbow agar for further testing and confirmation.

P3-125 Longitudinal Study of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Fresh Meat Processing Plant Environments

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Introduction: Small and very small fresh meat processing facilities have scarce resources to monitor foodborne pathogen contamination patterns and transmission dynamics in their premises. Environmental control of *Salmonella enterica, Escherichia coli* O157:H7, and *Listeria monocytogenes* is important to prevent cross-contamination of meat products by pathogens that may persist in a facility.

Purpose: The purpose of this study was to conduct a 6 month longitudinal study to monitor *Escherichia coli* O157:H7, *S. enterica* and *L. monocytogenes* contamination patterns and identify potential harborage sites in a small and very small fresh meat plant.

Methods: Both plants were sampled during mid-shift operation on a monthly basis. A total of 330 environmental sponge samples were collected from up to 55 sites (3 sponge swabs were collected to allow testing for each pathogen) in each facility. Samples collected included food contact surfaces (e.g., tables, scales, bins), non-food contact surfaces (e.g., walls, drains, sinks), and beef carcasses. Samples were microbiologically analyzed to isolate and detect *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes*, following modified versions of the U.S. Department of Agriculture

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Microbiology Laboratory Guidebook protocols 4.05, 5.05, and 8.07, respectively.

Results: *S. enterica* was isolated from 15 (4.5%) and 8 (2.4%) samples from Plant 1 and Plant 2, respectively. *S. enterica* was recovered more than once from 2 sites in Plant 1. *E. coli* O157:H7 was detected in 1.2% of samples in Plant 1, but was not recurrently isolated from a single site. *E. coli* O157:H7 was not isolated from Plant 2. *L. monocytogenes* was isolated from 17% of the samples from Plant 1 and 1.2% of samples from Plant 2. Recurrent isolation of *L. monocytogenes* from 15 sites was observed in Plant 1, while no single site in Plant 2 repeatedly tested positive for *L. monocytogenes*.

Significance: Our findings suggest that *L. monocytogenes* has higher prevalence than *S. enterica* and *E. coli* O157:H7 in the fresh meat processing plant environment. While *L. monocytogenes* may persistently contaminate the environment of fresh meat processing plants, *E. coli* O157:H7 and *S. enterica* contamination seems to be mostly sporadic.

P3-126 Comparison of Antimicrobial Properties of *Zanthoxylum armatum* and *Hibiscus sabdariffa* on Selected Foodborne Pathogens

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Introduction: Various reports showed that the incidence of foodborne diseases is a growing public health concern. Antibiotics resistance can lead to the emergence and dissemination of resistant strains of foodborne pathogens, which is ultimately passed onto consumers via food or through the direct contact with animals. Recent trend of utilizing natural compounds to inhibit foodborne pathogens is getting more attention among food scientists and food industries.

Purpose: In this experiment, we used two different types of phytochemicals (*Zanthoxylum armatum* and *Hibiscus sabdariffa)* in three different concentrations (5, 10 and 15%) to inhibit the growth of *Escherichia coli 0157:H7, Listeria monocytogenes, Salmonella enterica,* and *Staphylococcus aureus* by using disc diffusion method.

Methods: Bacteria stains were cultured in Tryptic Soy Broth and incubated overnight at 37 °C. The turbidity of each suspension was then adjusted to match that of a 0.5 McFarland standard: 1.5 X 10^8 CFU/ml. The suspension was then plated onto Muller-Hinton Agar (MHA) then allowed to dry at room temperature, then were place onto MHA that contain the bacteria. The discs were infused with 50 µl of two phytochemicals of the three concentrations (5, 10, and 15 %), then plates were allowed to dry for 45 minutes and were incubated overnight at 37 °C.

Results: Results of this experiment showed that 10 and 15% concentrations of both phytochemicals (*Zanthoxylum armatum* and *Hibiscus sabdariffa)* were more effective to reduce the growth of *E. coli, L. monocytogenes* and *S. aureus* compared to control. Results also indicated that 10% concentration of *Hibiscus sabdariffa* was more effective than *Zanthoxylum armatum*. Naturally, 15% concentration of both phytochemicals effectively inhibited the growth of above mentioned bacteria.

Significance: Natural extracts like *Zanthoxylum armatum* and *Hibiscus sabdariffa* can provide protection as promising antibacterial agents.

P3-127 Validation of a 24-hour Immunochromatographic Test Strip-based Method for the Detection of *Listeria* spp. on Environmental Surfaces

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Introduction: The USDA-FSIS has recently proposed a requirement for food processing establishments to withhold the release of product into commerce pending pathogen test results. For ready-to-eat (RTE) food processors, environmental monitoring of food contact surfaces for *Listeria* spp. is commonly used as an indicator of the potential for *L. monocytogenes* contamination. In order to reduce the time-to-result and therefore the cost of holding product pending test results, we have developed a simple, 24-hr, immunochromatographic test strip-based method for the detection of *Listeria* spp. on relevant environmental surfaces.

Purpose: The purpose of this study was to validate the performance of a new 24-hr, immunochromatographic test strip-based method for the detection of *Listeria* spp. on relevant environmental surfaces.

Methods: Environmental surfaces were spiked at levels ranging from 50 to 400 CFU per surface. A total of 120 spiked samples were tested by the SDIX method at 24 and 40 h and the USDA-FSIS cultural reference method. Inclusivity/exclusivity studies evaluated 50 *Listeria* strains and 35 non-*Listeria* bacterial strains.

Results: Total confirmed positives were 49, 54, and 48 for the SDIX 24 h method, the SDIX 40 h method, and the USDA-FSIS cultural reference method, respectively. Non-spiked samples from all environmental surfaces were reported as negative for *Listeria* spp. by all methods. The overall Chi square was 0.017 (P = 0.104) and 0.611 (P = 0.566) after a 24 h and 40 h enrichment, respectively, indicating that the test method was equivalent in performance to the reference method at both enrichment times. The method showed 100% sensitivity and 97% specificity for *Listeria* spp.

Significance: The new test method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria* species in the food processing environment and by doing so, minimize the contamination of food products by *Listeria monocytogenes*.

P3-128 Comparative Evaluation of the VIDAS® *Campylobacter* (CAM) Method for the Detection of *Campylobacter* from Selected Foods: AOAC Performance Tested Method℠ Validation Study

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Introduction: VIDAS® *Campylobacter* (CAM) is as an automated qualitative test for use on the VIDAS® family of instruments, for the detection of thermotolerant *Campylobacter* species in human food products and production environment samples using the ELFA technique (Enzyme Linked Fluorescent Assay).

Purpose: The purpose of this internal AOAC *Performance Tested Method℠* evaluation, conducted at Food Safety Net Services, was to compare the bioMérieux VIDAS® *Campylobacter* (CAM) method to the USDA/FSIS MLG and ISO reference methods for the detection of *Campylobacter* in chicken carcass rinses, turkey carcass sponges, raw pork, raw chicken breast, processed chicken nuggets.

Methods: The comparative food studies consisted of chicken carcass rinses, turkey carcass sponges, raw pork, raw chicken breast, and processed chicken nuggets. Twenty replicates of one fractional inoculation level and five uninoculated controls for each method and matrix were evaluated in this study. For the alternative method, samples were enriched in the proprietary ready-to-use broth, the CFB broth, for 48 hours at 42 °C prior to testing on VIDAS®. Microaerobic atmosphere was generated using a specific stomacher bag type, the combibag, including a microaerobic generator. This validation study also included the use of an agar plate, CampyFood Agar, for confirmation of CAM presumptive results.

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Results: Overall, the VIDAS® CAM method detected 66 confirmed positive samples versus 60 for the reference methods. The VIDAS® CAM test method was equivalent to the USDA method for the detection of *Campylobacter* in chicken rinsates and turkey swabs and equivalent to the ISO method for the detection of *Campylobacter* in raw chicken, processed chicken and raw pork, using the chi-square test at 5% level.

Significance: In the PTM validation study the VIDAS® CAM method demonstrated reliability as a rapid, qualitative method for the detection of *Campylobacter* in selected foods.

P3-129 Factors Contributing to the Transfer of *Escherichia coli* O157:H7 and *Listeria monocytogenes* between Cutting Surfaces and Fresh Produce; Cross-contamination Scenarios

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Introduction: Evaluation of bacterial transfer between biotic and abiotic surfaces, may contribute to our understanding on cross-contamination and assist to improve the hygienic practices during the preparation of fresh salads.

Purpose: To evaluate the transfer of *Escherichia coli* O157:H7 and *Listeria monocytogenes*: (i) from inoculated knives to three fresh leafy greens and (ii) from inoculated vegetables to knife and then to uninoculated vegetables.

Methods: Sterilized knives were inoculated with pathogen (6 log CFU/ml) and after 10 min (25 °C), knives were used to cut three vegetables (spinach, lettuce, cabbage). Three cuts were performed for every sample (20 g), with totally 10 samples (200 g) analyzed per knife. Inoculated (6 or 4 log CFU/g, 5 °C, 30 min) leafy greens (200 g) were cut with sterile knife 10 times, and the contaminated knife was then used to cut 600 g of uninoculated vegetable of the same type (30 cuts).

Results: Cell counts transferred from knives to vegetables were highly dependent on the type of salad and less on pathogen. During cut of the vegetables with a contaminated knife, 4.5-5 log CFU/g per cut for 30 cuts were observed for spinach, 2-4.5 log CFU/g per cut for lettuce and 0.7-3 log CFU/g per cut for cabbage, regardless of the pathogen. Contamination transferred from an inoculated lettuce to a sterile knife resulted in further transfer of cells to fresh uninoculated lettuce; 4 log CFU/g per cut of *E. coli* O157:H7 for high inoculum and 2 log CFU/g for low inoculum were observed during 30 cuttings, while the corresponding populations of *L. monocytogenes* were 2 log CFU/g and 0.5 log CFU/g. In case of cabbage, transfer occurred for high inoculum level with 2 log CFU/g per cut, but not for low inoculum level, for both pathogens.

Significance: The type of fresh produce has great impact on the bacterial transfer to knives, leading to high likelihood of cross-contamination in the industry of the preparation of salads.

P3-130 Comparative Evaluation of the CampyFood Agar (CFA) Method for the Selective Isolation and Enumeration of *Campylobacter* from Selected Foods: AOAC Performance Tested Method℠ Validation Study

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Introduction: CampyFood Agar (CFA) assay is a selective medium for the isolation, detection and enumeration of thermotolerant *Campylobacter* species, notably *C. jejuni, C. lari* and *C. coli*, from various foods.

Purpose: The purpose of this internal AOAC *Performance Tested Method℠* evaluation, conducted at Food Safety Net Services, was to compare the bioMérieux CampyFood Agar (CFA) assay to the USDA/FSIS MLG and ISO reference methods for the detection and the enumeration of *Campylobacter* in chicken carcass rinses, turkey carcass sponges, raw pork, raw chicken breast, and processed chicken nuggets.

Methods: The comparative food studies consisted of testing chicken carcass rinses, turkey carcass sponges, raw pork, raw chicken breast, and processed chicken nuggets. For the enumeration testing, five replicates of four inoculation levels (uninoculated, 50, 500 and 5,000 CFU/ml or g) for each method and matrix were evaluated for a total of 120 samples. The standard deviation was determined using log-transformed values and a T-test was performed.

For the detection testing, twenty replicates of one inoculation level and five uninoculated controls for each method and matrix were evaluated. Samples were enriched in the proprietary ready-to-use broth, the CFB broth, for 48 hours at 42 °C, before streaking onto the CFA agar. Microaerobic atmosphere was generated using a specific stomacher bag type, the combibag, including a microaerobic generator.

Results: Overall, the CFA method detected 66 positive samples versus 60 for the reference methods. The CFA test methods was equivalent to the USDA method for the detection of *Campylobacter* in chicken rinsates and turkey swabs and equivalent to the ISO method for the detection of *Campylobacter* in raw chicken, processed chicken and raw pork, using the chi-square test at 5% level.

Significance: In the PTM validation study the CFA method demonstrated reliability as a rapid method for the isolation, detection and enumeration of *Campylobacter* in selected foods.

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P3-131 Growth Characteristics and Development of a Predictive Model for *Escherichia coli* in Rice Cakes

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Developing Scientist Competitor

Introduction: Rice cake is a suitable food for microbial growth due to its high A_w, neutral pH, and storage temperature. However, a little study on microbiological safety of rice cakes has been performed.

Purpose: The objective of this study was to develop a predictive models for the growth of *Escherichia coli* in three kinds of rice cakes (*Garaetteok, Sirutteok*, and *Gyeongdan*) as a function of temperature (10-40 °C)

Methods: The growth data was fitted into the modified Gompertz model as primary models. The polynomial quadratic model was selected as secondary models and used the data such as specific growth rate (SGR), and lag time (LT) obtained from the primary models. The performances of predictive modeling were evaluated using mean square error (MSE), accuracy factor (A_f), bias factor (B_f) and coefficient of determination (r^2).

Results: The primary model of *E. coli* showed a good fit to Gompertz equation to obtain SGR and LT of each rice cakes. It was found that the values of SGR tends to increase in proportion to temperature and the value of LT decreased in inverse proportion to temperature in all three rice cakes. The appropriateness of secondary polynomial model was verified by a set of MSE, A_f, B_f and r^2. The MSE values of SGR and LT models in *Garaetteok* are 0.0006 and 0.28158, and r^2 are 0.9864 and 0.9962. The A_f values were 1.1628 and 1.3548, and B_f are 0.9484 and 0.9422, respectively. The MSE values of SGR and LT models in *Sirutteok* were 0.0008 and 0.0005 and r^2 are 0.9999 and 0.9999, respectively. The A_f values were 1.0575 and 1.0021, and B_f are 1.0575 and 0.9979. Lastly, the MSE values of SGR and LT models in *Gyeongdan* were both 0.000 and r^2 are both 1.000. The A_f values of SGR and LT models in *Gyeongdan* were 1.022 and 1.000, and B_f are 1.022 and 1.000, respectively.

Significance: These predictive models could be used for quantitative microbial risk assessment of *E. coli* in rice cakes.

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P3-132 Toxigenic *Clostridium difficile* in Retail Meats in Brazil

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Introduction: *Clostridium difficile* is an anaerobic, Gram-positive bacillus that causes diarrhea and pseudomembranous colitis in patients with previous antibiotic therapy. Three toxins are responsible for disease, toxin A encoded by the *tcdA*gene, toxin B encoded by *tcdB* gene and a binary toxin. Recently, researches have suggested that the disease may be associated with ingestion of contaminated foods of animal origin.

Purpose: The objective of this study was to evaluate the prevalence of toxigenic *C. difficile* in chicken, pork and beef acquired in retail markets in Campinas City, SP, Brazil.

Methods: The samples included 50 chicken, 30 beef and 35 pork. The samples were analyzed by enrichment in *Clostridium difficile* moxalactan norfloxacin (CDMN) broth at 37 °C for 10 days. After incubation, the broths were streaked onto CDMN agar and cycloserine cefoxitin fructose (CCF) agar, and submitted to alcohol shocked [alcohol at 1:1 (v/v), incubated at room temperature for 50 minutes and centrifuged] and the sediments were streaked onto CDMN agar and CCFA agar. The colonies were identified by PCR assays targeting the triose phosphate isomerase gene, *tpi*. The toxigenic profile was determined by PCR assay for genes *tcdA* and *tcdB* and by an enzyme immunoassay to detect the toxin A/B using the Ridascreen kit.

Results: *C. difficile* was detected in 8.7% (10/115) of the samples. Contamination frequencies were 18.0% (9/50) and 3.3% (1/30) in chicken and beef, respectively. *C. difficile* was not isolated from pork samples. Isolates of *C. difficile* from 3 chicken samples were toxin positive. *C. difficile tcdA+/tcdB+* was detected from 3 samples and the majority of the isolates was *tcdA-/tcdB-*.

Significance: The results showed the presence of toxigenic *C. difficile* in retail meats and the risk to occur the foodborne infection.

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P3-133 Disinfection of Shigatoxigenic *Escherichia coli* O157:H7 in Apple Cider and Skim Milk by Bacteriophages and Pulsed Electric Fields

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Introduction: With an increased number of foodborne outbreaks associated to Shiga toxin-producing *Escherichia coli* (STEC) in the last decade, growing interest in alternative disinfection methods has emerged. Bacteriophages and pulsed electric fields (PEF) are non-thermal decontamination techniques that could enhance food safety of liquid foods.

Purpose: In this study apple cider and skim milk (2% fat) were inoculated with Shiga toxin-positive *E. coli* O157:H7 and subsequently exposed to a cocktail of bacteriophages (CB), ambient PEF, heat-enhanced PEF (H/PEF) and combinations of these treatments.

Methods: Bacteriophages V10, V13, V15, and RV5 were combined in a cocktail for STEC inactivation in both liquid foods at 1:10 dilution, while both beverages were PEF-processed with electric field strengths of 38 kV/cm, for 1683 μs and at ≤45 °C, and H/PEF-treated with the same PEF processing conditions but a higher temperature level of ≤65 °C. Treated samples were stored at 4 °C for up to 42 days.

Results: In skim milk maximum STEC inactivation of 1.2, 1.7, 3.6, 1.5, and 3.7 log were achieved by CB, PEF, H/PEF, PEF/CB, and H/PEF/CB, respectively, while the same treatments led to corresponding STEC reductions of up to 2.2, 3.4, 4.6, 3.3, and 4.6 log in apple cider over the duration of the study. Both H/PEF and H/PEF/CB showed better efficacy for the reduction of the STEC ($P < 0.05$) than the other treatments.

Significance: The findings of this study show that H/PEF and H/PEF/P were the most effective treatments for STEC inactivation in both beverages, thus, representing promising liquid decontamination strategies. Disinfection with the non-thermal processing techniques had an overall greater impact on apple cider than in skim milk, thereby, suggesting that liquid foods with relatively high protein and fat contents may act as a greater barrier to the treatments' effectiveness than those with a comparatively high sugar composition.

P3-134 Growth Kinetics and Predictive Model of *Aeromonas hydrophila* in a Broth-based System

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Introduction: *Aeromonas hydrophila* has attracted attention as an emerging human pathogen because of its ability to grow at refrigeration temperatures like *Listeria monocytogenes* and *Yersinia enterocolitica*. *A. hydrophila* is neither salt (<5%) nor acid (min. pH 6) tolerant. It can be isolated from many kinds of foods including vegetables, meat (7), fish, seafood, raw milk, and chicken. *A. hydrophila* has a long survival time in the environment. Based on these reasons, *A. hydrophila* is of public health significance.

Purpose: Predictive food microbiology provides quantitative estimation of microbial growth in foods using mathematical modeling. This study provides a predictive model to describe the effect of temperature, pH and concentration of NaCl on the growth of *A. hydrophila* by response surface methodology (RSM). The model can be used as a reference in controlling *A. hydrophila* growth without the need for detection of the organism and may be of use for controlling growth. In this study, the growth characteristics of *A. hydrophila* were determined and a predictive model that could be used practically was developed.

Methods: This study was conducted to evaluate the survival characteristics and growth of *A. hydrophila* as a function of storage temperature (5 to 40 °C), pH value (6 to 8) and NaCl concentration (0 to 5%) with the aim of building a predictive model. The growth curves generated using a Gompertz equation and the relationship of the growth rate to the growth curves was modeled using a quadratic polynomial equation of RSM.

Results: *A. hydrophila* in TSB tended to grow well within a pH range of 6.0 to 8.0 and could not tolerate NaCl concentrations up to 5.0%. The interaction of pH and NaCl concentrations did not significantly affect the SGR. The primary model that we developed to obtain the SGR showed a good fit ($R^2 \geq 0.980$) with the Gompertz equation. A secondary polynomial model was obtained by non-linear regression analysis and calculated as: SGR model = $0.4577+0.0529X_1-0.1641X_2-0.1493X_3-0.0016X_1X_2-0.0001X_1X_3+0.0115X_2X_3 0.0006X_1^2+0.0114X_2^2+0.0150X_3^2$ (X_1=temperature, X_2=pH, X_3=NaCl). The appropriateness of the secondary polynomial model was verified by the mean square error (MSE = 0.0023), bias factor ($B_f = 0.922$), accuracy factor ($A_f = 1.343$) and coefficient of determination ($R^2 = 0.937$).

Significance: The model was found to be significant and the predicted values that were in agreement with previous studies. The model may be used as a reference in controlling *A. hydrophila* growth without the need for detection of the organism, but the model needs validation in a food environment before it can be practically applied. Therefore, it is necessary to develop effective control techniques to reduce the risk of *A. hydrophila* in food samples.

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P3-135 Growth Kinetics and Predictive Growth Model of *Aeromonas hydrophila* in a Squid-based System

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Introduction: Predictive modeling provides a fast and relatively cost-effect way to obtain reliable first estimates of microbial growth and survival. Predictive model was estimated *Aeromonas hydrophila* growth and to determine the shelf life of squid.

Purpose: This study investigated the growth characteristics of *A. hydrophila* on fresh squid and subsequently developed a predictive growth model of *A. hydrophila* on fresh squids as a function of storage temperature (5, 10, 20, 30, and 40 °C) using a response surface model (RSM). The model can be used as a reference in controlling *A. hydrophila* growth without the need for detection of the organism and may be of use for controlling growth.

Methods: Culture (1.0 x 10^2 cfu/g) as a cocktail of A. *hydrophila* (KCTC2358, KCTC12847, and KCCM11533) was inoculated on 5-10 spots on the surface of the squid. The squids were then stored at 5, 10, 20, 30, or 40 °C under aerobic conditions. The lag time and growth rate fitted to the modified Gompertz equation using a nonlinear regression model and the relationship of the lag time and growth rate to the growth curves was modeled using an RSM polynomial equation. The assessment of the RSM for describing the growth *A. hydrophila* of was evaluated using mean square error (MSE), bias (B_f) and accuracy factors (A_f).

Results: The *A. hydrophila* can grow on squid under refrigerated conditions, although the growth of the organism decreased at low temperatures. The primary models that we developed to obtain the specific growth rates (SGR) and lag time (LT) fit well (r^2 = 0.973-0.999) with the Gompertz equation. Secondary polynomial models were obtained by non-linear regression analyses and calculated as: SGR model = 0.05152 + 0.00337*T + 0.00039*T^2; and LT model = 50.51030 - 2.56290*T + 0.03446*T^2. The appropriateness of the secondary polynomial model was verified by the mean square error (MSE; 0.006 for the SGR model and 0.256 for the LT model), bias factor (B_f;0.999 for the SGR model and 1.007 for the LT model), accuracy factor (A_f; 1.025 for the SGR model and 1.026 for the LT model), and coefficient of determination (r^2; 0.991 for the SGR model and 0.993 for the LT model).

Significance: Our models may be of application fresh squid for manufacture of safe products by controlling *A. hydrophila* growth without the need for detection of the organism.

P3-136 Thermal Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Agona in Wheat Flour

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Introduction: Contaminated wheat flour has been identified as the probable vehicle of a multi-state outbreak of *Escherichia coli* O157:H7 associated with consumption of refrigerated, raw cookie dough. Several cookie dough manufacturers are using heated-treated flour for ready-to-bake products. Published data on thermal inactivation of foodborne pathogens in wheat flour remains scarce.

Purpose: To determine the effect of thermal treatment of artificially contaminated wheat flour on populations of *E coli* O157:H7 and *Salmonella* Agona.

Methods: *E. coli* O157:H7 and *Salmonella* Agona were added to wheat flour (1 g) in sterile plastic bags. Inoculated wheat was pulsified (15 s) to distribute cultures and pressed to a uniform thickness (1 mm). Bagged, inoculated wheat samples (approx. 9 log CFU/g) were submerged in a preheated shaking water bath for 1, 5, 15 or 30 minutes at 55, 60, 65 or 70 °C. Following thermal treatment, samples were submerged in ice water for 30 seconds and diluted with 0.1% peptone water. Samples (0.1 ml) were plated onto TSA and incubated at 37 °C for 24 h prior to enumeration.

Results: During heat treatment (30 min), populations of *E. coli* O157:H7 decreased by 2.9, 4.4, 5.7 and 5.7 log CFU/g, and *Salmonella* Agona populations decreased by 3.9, 4.3, 5.1 and 5.2 log CFU/g at 55, 60, 65, and 70 °C, respectively. Both pathogens remained detectable by direct plating or enrichment at 30 min of heat treatment.

Significance: Thermal processing of wheat flours may help improve the microbiological safety of ready-to-bake products.

P3-137 Genetic Diversity of *Listeria monocytogenes* Strains Recovered from the Food Sector in British Columbia, Canada

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* (*Lm*) is an environmentally ubiquitous pathogen and a frequent contaminant of ready-to-eat foods. Studies examining the prevalence of *Listeria* spp. in British Columbia (BC), Canada, found problems with *Lm* contamination in fish, and to a lesser degree in dairy and meat sectors. However, little is known about strain relatedness, and potential virulence risk of *Lm* in the BC food continuum.

Purpose: To assess diversity and virulence potential of *Lm* isolates recovered from BC's food chain.

Methods: Culture methods were used to recover *Lm* from food and environmental samples. Isolates were serotyped and subjected to pulsed-field gel electrophoresis (PFGE). Based on PFGE, premature stop codons (PMSC) within *inlA* were screened by DNA sequencing in 56 unique isolates.

Results: In total, 111 *Lm* isolates were recovered from three dairy, seven fish and five meat facilities. Isolates serotyped as 1/2a (42%), 4b (37%), 1/2c (12%), 1/2b (5%), and 3a (4%). PMSCs in *inlA* were observed in 36% of isolates, including eight 1/2b, six 1/2a, four 3a, and one 1/2b and 4b *Lm* serotype. Conversely, no PMSCs were seen in 64% of isolates (20 4b, 15 1/2a and one 1/2b), though 23% possessed a previously unreported three-codon deletion in positions 738-740. PFGE revealed 36 unique pulsotypes; closely related patterns were observed in dairy and meat, fish and meat, but not dairy and fish facilities. More than one *Lm* serotype and pulsotype were seen in 54% and 69% of the facilities, respectively.

Significance: Genetically diverse *Lm* strains were observed across different food facilities. Most strains belonged to listeriosis-causing serotypes encoding a full-length InlA protein required for disease. Accordingly, *Lm* recovered along the BC food continuum, and specifically fish, may pose high risk to the consuming public.

P3-138 Detection of Seven Top STEC *Escherichia coli* Serotypes by the Assurance GDS Top STEC MPX Assay

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Introduction: The United States Department of Agriculture (USDA) has proposed new regulations applicable to the analysis of certain raw beef products which define six Shiga-toxigenic *E.coli* serotypes, in addition to O157, ("Top 7 STEC") as adulterants. The Assurance GDS Top STEC MPX assay has been developed to provide an effective testing solution to satisfy this requirement. System components include Top 7 STEC

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serotype-specific immunomagnetic sample concentration reagents with the PickPen concentration device, amplification and identification reagents defining STEC genes *eae*A (O157), *eae*A STEC, *stx*1 and *stx*2, with Internal Control in a multiplex format; and microbiological confirmation reagents for Top 7 STEC isolates.

Purpose: To define the specificity (Inclusivity and Exclusivity) of the Assurance GDS Top STEC MPX assay for *E.coli* serotypes O26, O45, O103, O111, O121, O145, and O157.

Methods: Inclusivity was determined by analyzing a panel of 67 STEC strains representing all seven Top STEC serotypes. Exclusivity was determined by analyzing a panel of 31 strains representing potential cross-reactive microorganisms.

Results: Sixty-seven (67) *E.coli* Top 7 STEC inclusivity isolates were correctly identified and typed for the *eae*A and *stx* genes by the MPX assay, with correct differentiation of *eae*A (O157), and *eae*A (STEC) genes. All 31 Exclusivity panel non-Top STEC strains gave negative results.

Significance: These results verify the accuracy of the Assurance GDS Top STEC MPX assay for the identification of Top 7 STEC organisms.

P3-139 Validation Study of Assurance GDS MPX for Top STEC in Raw Beef Products

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Introduction: Raw beef products have been analyzed for the presence of *E.coli* O157:H7 as defined in the United States Department of Agriculture (USDA) regulations for several years. More recently, the USDA has proposed a new requirement to test certain raw beef products for a broader group of *E. coli* commonly referred to as "the top six Shiga toxigenic *E.coli*" or "Top STEC" as adulterants. The USDA considers the following six STEC O serotypes, in addition to O157, to represent the greatest public health risk: O26, O45, O103, O111, O121 and O145. The USDA will require routine testing of all these 7 serotypes for raw beef starting in March 2012. Assurance GDSTop STEC MPX is an assay that allows simultaneous detection of *E.coli* O157:H7 and also the top 6 non-O157 STEC (Top 7 STEC) in a sample.

Purpose: Develop an assay to simultaneously detect *E.coli* O157:H7 and the top 6 non-O157 STEC to fulfill new USDA regulations.

Methods: Raw beef (beef trim, ground beef and finely textured beef) samples were inoculated with a 2-day cold stressed target organism. 375 g samples were analyzed with the Assurance GDS Top STEC MPX method for determination of the simultaneous presence of *E.coli* O157:H7 and the top 6 non-O157 STEC. All samples were confirmed via IMS plating on a modified Rainbow agar and BVCC agar.

Results: All seven serotypes were analyzed among all three food types. A total of 198 raw beef samples were inoculated with 0.5 – 0.7 CFU per sample to achieve partial recovery. The enrichments were assayed with Assurance GDS Top STEC MPX and confirmed using IMS onto selective agar plates. 114 samples were positive by Assurance GDS MPX after 10 hours. There was one sample which was negative by Assurance GDS Top STEC MPX which confirmed positive, for an overall detection rate of 99%.

Significance: The Assurance GDS Top STEC MPX assay is a reliable method for simultaneous detection of both *E.coli* O157:H7 and Top 6 non-O157 STEC in a common sample.

P3-140 Influence of Vacuum Cooling on Microbe Infiltration in Fresh Leafy Greens

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Introduction: Internalization of bacteria in fresh produce is a major food safety risk factor as internalized bacteria are refractory to most washing and sanitation procedures. Vacuum cooling has been identified as one of processing steps that can enhance internalization of microbes in fresh produce. Currently there is limited insight into what factors during vacuum cooling influence internalization of microbes?

Purpose: The aim of our study was to elucidate the risk of internalization of surface inoculated *E. coli O157:H7* upon vacuum cooling of lettuce as a function of microbial inoculation (high: 6 log CFU per leaf disk) or low: 3 log CFU per disk), surface moisture, and on both sides (abaxial and adaxial side) of lettuce leaves. To measure internalization of microbes in intact leafy greens, multiphoton 3-D microscopy was used. This novel microscopy approach addresses the challenges of autofluorescence and limited depth penetration to enable 3D microscopy of intact leaves.

Methods: Commercial lettuce purchased from a grocery store was washed, and the leaf surface was sprayed evenly with a solution of *E. coli* O157:H7 GFP to inoculate the surface of lettuce. After vacuum cooling treatment, the lettuce leaves were imaged with multiphoton microscopy. For quantitative and statistical analysis, the number of microbes associated with stomata and infiltrated into the leaf was quantified.

Results: Results based on imaging measurements demonstrated that the vacuum cooling process does not significantly increase the risk of internalization ($P > 0.5$) of surface inoculated E. *coli* into an intact lettuce leaf. The imaging results also show that the vacuum cooling process increased the number of bacteria associated with stomata ($P < 0.5$). These results were observed for both high moisture and low moisture conditions for samples inoculated on abaxial surface of lettuce leaves.

Significance: The imaging measurements highlight that the vacuum cooling process does not significantly increase the risk of internalization of microbes.

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P3-141 Evaluation of Virulence Profiles of Environmental Avian Pathogenic *Escherichia coli* O157 Isolates

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Introduction: Bacterial pathogens may pick up virulence factors that alter their phenotype. Such was the case with the novel STEC *E. coli* O104:H4 which caused a large outbreak associated with sprouted seeds. Avian pathogenic *E. coli*(APEC) may contain an array of virulence factors. Poultry litter is often composted to be used as fertilizer for agricultural crops and fields. Because poultry litter and poultry may be reservoirs of APEC, this may be important for public health and food safety.

Purpose: The pathogenic mechanisms of APEC which cause disease in poultry may also contain virulence factors important to causing human infections. An avian model was used to assess the relationship between clinical phenotypes and the genotypic virulence profiles of APEC isolates. Fecal shedding by APEC was determined, which is important given APEC are traditionally a respiratory problem in broilers.

Methods: Two-week old broilers were orally inoculated with 8 O157 APEC isolates containing genes important for protease activity, hemolysis, and attachment (*ehxA*, *espP*, *katP*, *stcE*). Cloacal swabs were collected over 4-weeks post-inoculation. Fecal shedding of APEC was quantified on Sorbitol MacConkey agar (SMAC) and confirmed by molecular detection. Control birds were sham inoculated with TSB.

Results: Birds did not shed APEC pre-inoculation, but all 8 groups (n=24) were positive for fecal-shedding of APEC by day 27 post-inoculation. *E. coli* strains with *ehxA* were shed at 2 dpi on the scale of 10^6 CFU. Hemolysin activity appeared most important as determined by PCR on virulent APEC strains. Necropsies on deceased birds or those culled due to clinical illness showed signs of extreme hemolysis. APEC

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O157 isolates showed varying clinical phenotypes in birds. Symptoms included ruffled feathers, labored breathing, bloated intestines, hemorrhaged breast muscle, ascites, and diarrhea.

Significance: APEC that are *stx* negative were extremely virulent in broilers and appear to be shed in feces. APEC genotype may not dictate phenotypic profiles as seen here in live birds. This is being assessed in Caco-2 cells as well.

P3-142 Influence of Vitamin Exposure on the Expression of Selected *Escherichia coli* O157:H7 Stress Response Genes

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Introduction: The fortification of foods ensures a daily source of vitamins in our diet. Additionally, certain vitamins are produced by our enteric flora. It is possible that food-related or enterically-produced vitamins are triggers for the expression of the genes related to bacterial survival in the competitive enteric environment.

Purpose: To determine whether vitamins influence the expression of stress response genes in *Escherichia coli* O157:H7.

Methods: *E. coli* O157:H7 EDL933 carrying *rpoS-lux* or *osmC-lux* constructs were treated with optimized concentrations of biotin, folate, cobalamin, pantothenate, riboflavin, menaquinone ascorbate and α-tocopherol. Cells were screened for bioluminescence during 5.5 h exposure, and compared to controls. Additionally, three *E. coli* O157:H7 EDL933 were treated with the maximal concentrations of respective vitamins and plated on Luria Bertani agar to assess growth.

Results: Following vitamin addition, repression in relative bioluminescence was observed with cobalamin (1 mg/ml, >8-fold, *rpoS*; >13-fold, *osmC*) and 2-fold induction with α-tocopherol (1 mg/ml, *rpoS*) and menaquinone (0.005, 0.05 mg/ml, *osmC*), while after 1 h treatment, >2-fold repression resulted with pantothenate (50 mg/ml, *rpoS* and *osmC*) and ascorbate (10 mg/ml, *osmC*), and >3-fold repression with ascorbate (10 mg/ml, *rpoS*). After 5.5 h, previous inductions of α-tocopherol (*rpoS*) and menaquinone (*osmC*) were ameliorated to <2-fold, strong repression by ascorbate (18-fold, *rpoS* and >83-fold, *osmC*), pantothenate (>224-fold, *rpoS* and >216-fold, *osmC*), and cobalamin (>14-fold, *rpoS* and >6-fold, *osmC*) occurred, and 2-fold repression for pantothenate (5 mg/ml, *osmC*) resulted. Following vitamin exposure, significantly fewer bacteria were observed in pantothenate (50 mg/ml, 1.5 and 3 h; $P < 0.001$) and ascorbate [10 mg/ml, 1.5 h ($P < 0.05$) and 3 h ($P < 0.001$)] treatments, while significantly more *E. coli* O157:H7 were observed in biotin (0.1 mg/ml, 1.5 h; $P < 0.05$) and cobalamin (1 mg/ml, 3 h; $P < 0.05$) treatments when compared to controls.

Significance: Our results show vitamins present in food or those produced by enteric bacteria influence *rpoS* and o*smC* gene expression of *E. coli* O157:H7.

P3-143 Influence of Vitamins on *Escherichia coli* O157:H7 Adherence to HeLa cells

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Developing Scientist Competitor

Introduction: Whether consumed through diet, supplementation, or enterically-produced, vitamins are common constituents in our enteric environment. Other constituents of the human enteric system, including bicarbonate and hormones, are known to influence pathogenic processes, though no data regarding vitamin impact on these exist.

Purpose: Examine the impact of vitamin exposure on adherence of *Escherichia coli* O157:H7 to HeLa cells.

Methods: Three independent *E. coli* O157:H7 cultures were exposed to optimized concentrations of enterically produced (biotin, folate, riboflavin, menaquinone, pantothenate and cobalamin) or food-related vitamins (ascorbate, α-tocopherol). At 1.5 and 3h, cells were plated on Luria Bertani agar or screened for adherence/invasive capacity using HeLa cells, and compared to respective controls.

Results: Following vitamin exposure, significantly fewer bacteria were observed in pantothenate (50 mg/ml, 1.5 and 3h; $P < 0.001$) and ascorbate [10 mg/ml, 1.5 ($P < 0.05$) and 3h ($P < 0.001$)] treatments, while significantly more *E. coli*O157:H7 were observed in biotin (0.1 mg/ml, 1.5h; $P < 0.05$) and cobalamin (1 mg/ml, 3h; $P < 0.05$) treatments when compared to controls. Following 1.5h exposure, significantly increased adherence to HeLa cells was observed for ascorbate (0.1 mg/ml; $P < 0.05$), pantothenate (5 mg/ml, 50 mg/ml; $P < 0.001$) and cobalamin (0.01, 0.1, 1 mg/ml; $P < 0.001$) treatments whilst exposure to folate (0.001 mg/ml; $P < 0.001$) and biotin (0.1 mg/ml; $P < 0.05$) reduced adherence. At 3h exposure, significantly increased adherence was observed for biotin (0.01, 0.1mg/ml; $P < 0.05$), riboflavin (0.0001, 0.001, 0.01 mg/ml; $P < 0.001$, $P < 0.01$, $P < 0.001$, respectively), pantothenate (5, 50 mg/ml; $P < 0.001$), cobalamin (0.01, 0.1, 1 mg/ml; $P < 0.001$) and α-tocopherol (1 mg/ml; $P < 0.001$).

Significance: Our results show vitamins may influence the adherence capacity of *E. coli* O157:H7 to HeLa cells, suggesting a possible role as an environmental cue in the intestinal environment. As such, further work is required to quantify the manner by which vitamins associated with us or our food influence stress and virulence processes in *E. coli* O157:H7.

P3-144 Surface Survival and Internalization of *Salmonella* through Natural Cracks on Developing Cantaloupe Fruit, Alone or in the Presence of the Plant Pathogen *Erwinia tracheiphila*

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Introduction: *Salmonella* Poona (SP) can contaminate cantaloupes, but whether bacteria internalize into edible tissues or other microflora affect invasion, are unknown.

Purpose: We hypothesized that natural cracks formed during cantaloupe net initiation facilitate SP internalization. We investigated whether SP survives on the rind and internalizes through these cracks, either alone or with the wilt pathogen *Erwinia tracheiphila* (Et).

Methods: Drops (20µl; 10^7 CFU/ml) of an SP-Et mixture, or of each pathogen alone, were brushed onto newly formed fruit cracks. Rind or underlying mesocarp samples were assayed by direct and enrichment plating at 0, 9 and 24 days post-inoculation (DPI).

Results: Fruits inoculated with Et (61%) or SP+Et (56%) developed watersoaked lesions. Et was recovered from 80% and 20% of rinds inoculated with Et or SP+Et, respectively, at 0 DPI, but 100% of rinds having lesions yielded Et at 9 and 24 DPI. 25% of mesocarp samples of the same fruits were Et+. SP recovery from rinds inoculated with SP or SP+Et was 7.6×10^2 and 9.2×10^2 cells/ml, respectively, at 0 DPI, but dropped to 1.7 and 2.9 cells/ml (9 DPI) and 0 and 1.6 cells/ml (24 DPI). SP survived longer on fruits co-inoculated with Et, and on fruits with lesions, than on those of other treatments. None of 67 mesocarp samples from fruits receiving SP or SP+Et were SP+. Neither SP nor Et was found on any

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non-inoculated fruits.

Significance: SP, introduced onto cracks of young cantaloupe fruits, was later recovered from rind surfaces but never from edible mesocarp. SP survived longer on rinds in the presence of Et, suggesting that community microbes influence fitness. Et also survived on the rind but, unlike SP, Et frequently invaded the mesocarp, causing watersoaked lesions. Interestingly, although Et's usual plant niche is xylem, neither it nor SP was detected in any non-inoculated fruits, suggesting a lack of systemic movement.

P3-145 Prevalence and Levels of *Listeria monocytogenes* (Lm) in Ready-to-Eat Foods (RTE) at Retail

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Introduction: Although significant efforts have been taken to control *Lm* in RTE foods over the last decade, a well-designed survey is needed to determine whether changes occur in the "true" prevalence and levels of the pathogen and to provide current data to assess the relative ranking of higher risk foods.

Purpose: A multi-agency, multi-disciplinary study was undertaken to determine the current prevalence and levels of *Lm*in deli-packaged versus prepackaged RTE foods purchased at retail establishments in four FoodNet sites.

Methods: Phase I of the study included ten product categories: smoked seafood, seafood salad, low acid cut fruits, soft cheese, deli salads (non-meat), raw milk, sandwiches, deli meats, deli salads containing meat, and dried/fermented sausage. Samples were collected in both supermarket chain and independent grocery stores in California, Maryland, Connecticut and Georgia over a 12-month period beginning December 2010. Collaborations between FDA, FSIS, ARS and several universities enabled the collection of a representative and sufficient number of samples. Samples were analyzed using the FDA-BAM method, which includes screening (25 gram or ml per each sample) and enumeration of positive samples by the MPN method and direct plating.

Results: Of the ca.7,500 FDA regulated samples tested to date, the observed prevalence ranged from ca. 0% to 1.0% for seven product categories. The prevalence data for the ca. 3,500 FSIS-regulated products are currently being analyzed. For the 43 samples testing positive during screening, *Lm* levels ranged from ca. <0.3 MPN/g to 2.4 log CFU/g.

Significance: This is the most comprehensive survey of *Lm* in retail RTE foods in the past decade. Our findings provide data to assess changes in *Lm* prevalence and levels in RTE foods and will be used to update the 2003 Interagency *Lm*risk assessment. The study also underscores the importance of continued research to develop and validate interventions to ensure a wholesome food supply.

P3-146 Understanding the Role of the Catalase/peroxide Genes in H_2O_2 Resistance of *Escherichia coli* Serotype O157:H7 Biofilms

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Introduction: *Escherichia coli* serotype O157:H7 defenses against H_2O_2 include the peroxiredoxin AhpC and three catalases: KatG (catalase-peroxidase), KatE (catalase), and the plasmid-encoded KatP (catalase/peroxidase). AhpC, KatG, and KatP are induced by OxyR in exponential phase, while KatE is induced by RpoS and independent of OxyR. In stationary phase, basal expression of KatG and AhpC are also maintained by RpoS.

Purpose: In this study we investigated the role and regulation of each catalase/peroxidase in the H_2O_2 resistance of a biofilm forming variant of strain EDL933 (43895OR) residing in a single-species biofilm.

Methods: We constructed mutants of isolate 43895OR with deletion of *rpoS*, *oxyR*, both *oxyR*/*rpoS*, or with deletions of 3 of the 4 peroxide scavenging enzymes. Strains bearing only *katG*, *katP*, *ahpC* or *katE* were compared to a strain with deletion of all four genes and to the wild-type for survival differences following H_2O_2 challenge. *rpoS* deletions constructed in each of the strains bearing a single catalase/peroxidase gene defined the regulatory influence of RpoS.

Results: Strain 43895OR survival was greater ($P < 0.05$) in biofilm than in planktonic cells, and full resistance required *rpoS* but not *oxyR*. In 72-h biofilms, *katG* and *katE* provided the most protection, with *katG* able to maintain full resistance. Each mutant bearing a single resistance gene survived under reduced O_2 conditions, but their counts were significantly lowered ($P < 0.05$) in the absence of RpoS. In contrast, RpoS deletion was lethal in a strain missing all four resistance genes indicating that all four resistance genes provide some RpoS-independent peroxide resistance in biofilms.

Significance: This is the first study of the role and regulation of the major peroxide resistance genes in *E. coli* serotype O157:H7 biofilms. Understanding the mechanism and regulation of peroxide resistance in biofilms will aid in developing intervention strategies for the control of *E. coli* O157:H7.

P3-147 Laboratory Performance on the Recovery of *Listeria monocytogenes* in Queso Fresco Cheese and Alfalfa Sprouts

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Introduction: The proficiency testing (PT) program at the Institute for Food Safety and Health has an established infrastructure to produce samples tailored to specific needs for testing laboratory performance. Laboratory performance is an important aspect of effective food safety testing.

Purpose: Laboratories were instructed to isolate *Listeria monocytogenes* from samples of queso fresco cheese or alfalfa sprouts. Each food matrix represented a separate PT exercise that consisted of 5 samples each.

Methods: Fifteen laboratories received inoculated queso fresco cheese in which samples 1 , 3, and 5 contained *L. monocytogenes* (7500 – 9800 CFU/g), 3 and 5 also contained *L. grayii* (600 CFU/g) and *L. innocua* (500 CFU/g), respectively, and 2 and 4 had no added inoculum. In a separate exercise, sixty-one laboratories received inoculated alfalfa sprouts in which samples 1, 2, and 5 contained *L. monocytogenes* (200 CFU/g), sample 2 also contained *L. grayii* (200 CFU/g) and 5 also contained *L. innocua* (200 CFU/g). Samples 3 and 4 both contained 200 CFU/g each of *L. grayii* and *L. innocua* only.

Results: All participants (100%) isolated *L. monocytogenes* from inoculated queso fresco cheese samples. Although not required 27% identified *L.grayii* and 67% identified *L. innocua*. In the alfalfa sprout samples, 92% of participants correctly identified *L. monocytogenes*. In addition

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to *L. monocytogenes* participants detected confounder organisms in the following samples: 2, *L. grayii* (15%); 3, *L. innocua* (41%) and *L. grayii* (20%); 4, *L. innocua* (44%) and *L. grayii* (16%); and 5, *L. grayii* (10%).

Significance: For both exercises a high percentage of laboratories correctly identified *L. monocytogenes* in food matrices that can be difficult to analyze. Laboratories that participate in PT exercises are able to demonstrate their ability to detect pathogens effectively and demonstrates the need for a PT program that can create samples tailored to specific needs.

P3-148 Performance Assessment of a New *Cronobacter* spp. Detection Method in Infant Formula and Environmental Samples

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Introduction: The iQ-Check™ *Cronobacter* spp method is a new *Cronobacter* spp detection method based on Real-Time PCR principle. Flexibility is offered:

- in the DNA extraction step, since the users can choose between the standard and easy protocol;
- by storing the enrichment broth at 5 ± 3 °C for 3 days, before running the PCR analysis.

Purpose: An independent study was conducted to validate this new method in comparison to the ISO/TS 22964 reference method, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The method protocol includes an overnight enrichment in BPW supplemented with vancomycin. An additional sub-culture is done in BPW for 4h ± 1h for infant formula analysis. After the DNA extraction step, real-time PCR is run with a Bio-Rad automate. The presumptive positive results are confirmed by direct streaking onto RAPID'Sakazakii Agar for infant formula, and after a subculture in mLST prior to streaking for environmental samples

Results: 171 infant formula and environmental samples were analyzed in the relative accuracy, sensitivity and specificity study, which concludes to equivalent performances between the new method *Cronobacter* spp and the ISO/TS 22964 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the *Cronobacter* spp new method vary from 0.5 et 1.6 CFU/25 g, those of the ISO standard from 0.5 to 1.5 CFU/25 g. The selectivity and specificity of the alternative method was assessed by testing 52 target strains and 31 non-target strains.

Significance: The new method is a reliable alternative method for *Cronobacter* spp detection in infant formula and environmental samples, and offers important economic savings by reducing time to result and handling time.

P3-149 Development of a New Device for the Rapid Detection of Aciduric Microorganisms

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Introduction: A new Soleris® vial was developed to rapidly detect aciduric microorganism contamination in high-acid food products. The vial, based on detection of carbon dioxide produced from the metabolism of the organisms, consists of a detection chamber containing carbon dioxide indicators separated by a barrier layer from a chamber containing a growth medium and test sample. The majority of aciduric microorganisms can be detected within 48 hours. The vial is used in conjunction with the instrument system.

Purpose: The purpose of this study was to develop and assess the performance of a device for the rapid detection of aciduric microorganisms in high-acid food products in the juice and beverage industries.

Methods: Experiments were performed to select the optimal growth medium and semi-permeable matrix indicator chemistries that would produce the most rapid detection of aciduric microorganisms in the new vial. Orange Serum Broth (Acumedia) was selected as the growth medium. This medium was optimized for growth of the target organisms. Inclusivity studies were performed using a panel of the target organisms at levels of <100 CFU/ml. In addition, the detection time in the instrument and growth of low inoculum levels of the organisms in food matrices was examined.

Results: The inclusivity test panel of twenty-one organisms consisted of bacteria including *Lactobacillus*, *Leuconostoc* and *Alicyclobacillus*, yeasts and molds. The instrument detection times in juice products were as low as 20 hours for an inoculum level of 3 CFU/ml for *Leuconostoc mesenteroides*, 33 hours for an inoculum level of 9 CFU/ml for *Aspergillus niger* and 16 hours for an inoculum level of 39 CFU/ml for *Candida krusei*. The majority of the other target organisms containing < 100 CFU/ml detected in < 48 hours. The acidic food matrices tested did not interfere with the vial test.

Significance: The new Orange Serum vial provides a system for the rapid detection of aciduric microorganisms in many high-acid food products in < 48 hours compared to the standard incubation time of 5 days for agar plates.

P3-150 Novel Automated Workflow Reducing Time to Result for Detecting O157:H7 and Non-O157 STEC Strains from 375 g Beef Samples

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Introduction: Rapid detection of low levels of pathogenic microorganisms in food by molecular methods is often difficult due to the limited amount of targets, complexity of food matrices and presence of PCR inhibitors. We have developed a new workflow for detection of *E. coli* O157:H7 and 6 non-O157 STECs in 375 g beef samples in less than 8 hours of total Time-to-Result (TTR). The workflow includes an automated sample preparation method and rapid real-time PCR detection of O-group specific genetic targets as well as *stx-1/stx-2* and *eae* genes.

Purpose: To design an effective automated workflow for detecting at least 1 CFU of pathogenic *E.coli* in 375 g of ground beef and beef trim samples in one 8 hrs working shift.

Methods: 375 g ground beef and beef trim samples were spiked with ~1 CFU of *E. coli* O157:H7 and non-O157 STECs. After 6.5 hours of enrichment in optimized culture conditions, samples were processed using fast Immuno Magnetic Separation (IMS) sample preparation protocol automated on the MagMAX™ Express-96 Magnetic Particle Processor. The qPCR analysis of purified samples was performed on 7500 Fast Real-Time PCR System under fast cycling conditions. The results of detection were confirmed using plating and overnight enrichment of same samples followed by analysis with AOAC-validated protocols.

Results: 100% correlation was obtained between real-time PCR results and control methods. The total time of the workflow was slightly under 8 hours. The sample preparation method was highly efficient in capturing of target microorganisms and removal of PCR inhibitors, as demonstrated by short TTR and robust detection of the internal positive control included in the assay. The optimized workflow has minimal number of liquid handling steps that reduces the risk of operator error and cross contamination between samples.

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Significance: The ease of use, rapidity and efficiency of our novel IMS-based sample preparation workflow in combination with real-time PCR allow detecting low amounts of pathogenic *E.coli* strains in difficult food matrices such as 375 g of ground beef in a reduced timeframe.

P3-151 An Effective Real-Time Quantitative PCR Protocol for Quantification of Pathogens in Foodstuffs

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Developing Scientist Competitor

Introduction: Several methods of real-time quantitative PCR have been developed for the detection and quantification of pathogens in food products. All molecular techniques needed an enrichment step which could hedge the quantification. Furthermore, most of those techniques are not available on a large range of foodstuffs.

Purpose: The behavior of our study was to develop an effective molecular quantification for pathogens, as *Listeria monocytogenes*, *Escherichia coli* O157:H7 or *Salmonella* spp. To present an interest for food industries, our technique should be efficient on several foodstuffs.

Methods: Experiments were carried out on pure culture, pork minced meat, white pudding, ham, and different field pancakes. Food matrices have been artificially contaminated with 4 to 100 cfu/g of *Listeria monocytogenes*, *Escherichia coli*O157:H7 or *Salmonella* spp. During the same period, to numerate pathogens, classical microbial analysis and molecular quantification by real-time quantitative PCR had been carried out at + 20 °C and with aging tests. Taqman technology was used for pathogens quantification.

Results: Different standard curves were established for each pathogen group. Those were carried out and validated in pure culture, and also in the different foodstuffs. Statistical analyses between classical and molecular quantifications allowed the validation of standard curves. The lower threshold for quantification was about 5 ufc/g. Under this threshold, detection was still possible.

Significance: Many studies have confirmed the value of qPCR as a rapid and reliable method that could be used in food industries for detecting organisms of interest, but there are still reservations about its routine use in food analysis. Commercial kits have been developed for the detection, but not for precise quantification of the main pathogens. Our technique allowed an effective quantification of three groups of pathogens, at least for now. This new technique could be used directly on food matrix, without enrichment step, with a vey interesting lower quantification threshold.

P3-152 Design and Evaluation of a Real-Time PCR Method for Detecting O157:H7 and Non-O157 STEC Strains from Beef Samples

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Introduction: *E. coli* O157:H7 was first recognized as a human pathogen in 1982 and until recently was the only *E. coli* strain mandated for testing by the USDA. In late 2011, the USDA announced it will declare 6 additional Shiga toxin-producing *E. coli* serotypes as adulterants, namely O26, O45, O103, O111, O121, and O145. These 6 non-O157 STECs will be classified as adulterants if they also contain the virulence genes, *eae* and stx_1 and/or stx_2.

Purpose: To design a real-time PCR assay for detecting STEC adulterants including O157:H7 and the 6 non-O157 STEC bacteria using multiplex designs to reduce the number of tests to one screening assay and one confirmation assay.

Methods: A minimum of four TaqMan® real-time PCR assays were designed against each of the 6 non-O157 STEC serotypes using Applied Biosystems assay design software. Additional assays were also designed against virulence factorsstx_1, stx_2, and *eae*. Each assay was tested against an inclusion/exclusion panel of 242 *E. coli* strains and 31 non-*E. coli* bacteria to determine assay specificity and sensitivity. The inclusion panel included all known *E. coli* O-types, strains belonging to various STEC serogroups, and 62 of the big six non-O157 STEC strains. High throughput screening by real-time PCR was performed on the 7900 real-time PCR system and multiplex optimization was performed on the 7500 Fast real-time PCR system using standard cycling conditions (95 °C for 10 min, followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 60 seconds).

Results: Multiple assays for each of the 6 non-O157 STECs detected all inclusion strains within the targeted serotype, and no exclusion strains from other serotypes. The *stx* assays detected all variants of stx_1 and stx_2 tested including $stx_{2f}$and stx_{2g}. The assays were combined into two separate multiplex assays and optimized using statistical methods based on Design of Experiments. The assays will be evaluated for detecting STEC in ground beef and beef trim.

Significance: Regulations are moving toward increased testing for foodborne pathogens. Multiplex real-time PCR can combine up to four PCR tests into a single reaction by using four different fluorescent dyes, reducing time and costs.

P3-153 Validation of a New Method According to the ISO 16140 Standard for the Next-day Detection of *Listeria* spp. in Food Products and Environmental Samples

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Introduction: The VIDAS® UP *Listeria* (LPT) assay is a specific phage protein ligand assay performed in the automated VIDAS instrument associated with a 1-step enrichment procedure.

Purpose: A study was conducted by the independent Expert Laboratory Eurofins IPL Nord, to validate this new method, as part of the NF Validation approval process.

Methods: Samples were enriched at 30 ± 1 °C for 26 hours (food samples) or 22 hours (environmental surfaces) in the ready-to-use proprietary broth, the LPT broth. After incubation, samples were boiled for 5 ± 1 minutes before performing the assay. All presumptive positive samples were further confirmed after streaking on PALCAM or on a chromogenic agar according to Ottaviani Agosti. This new method was compared to the ISO 11290-1/A1 reference method, according to the ISO 16140 standard.

Results: A comparative study was performed on 349 products distributed over the 5 categories meat, dairy, vegetable, seafood and environmental samples of which 69% were naturally contaminated. The phage method detected 160 positive samples compared to 162 for the reference method. There was no statistical difference between the two methods using the Mc Nemar test at 5% level. The 50% detection limit was determined on 5 different products/strains combination tested at 4 contamination levels. Results were comparable between the two methods. In the inclusivity study, all the 51 *Listeria* spp strains tested were detected and in the inclusivity study, none of the 30 non-*Listeria* strains gave a positive result.

Significance: The LPT method provides a simple, convenient and reliable method for detection of *Listeria* species in food and environmental samples, providing a presumptive result for the presence of *Listeria* in less than 23 hours for environmental surfaces and 27 hours for food samples.

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P3-154 Selection of Aptamers against *Salmonella enterica* Serovar Typhimurium

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Introduction: Salmonellosis is one of the most frequently reported bacterial foodborne diseases and is a major economic and public health issue worldwide, which is caused by multidrug resistant (MDR) strains such as *Salmonella enterica*serovar Typhimurium or *S. enterica* serovar Newport. There is a real need, therefore, to develop alternative molecular approaches for identifying *S.* Typhimurium.

Purpose: In this work, we describe the selection of aptamers against whole *S.* Typhimurium bacterial cells, which is different from aptamers recognize *S.* Typhimurium outer membrane proteins reported by Joshi et al in 2009.

Methods: A high-affinity ssDNA aptamer binding to *Salmonella enterica* serovar Typhimurium was obtained by a whole bacterium-based SELEX procedure. After nine rounds of selection with *S.* Typhimurium as the target, a highly enriched oligonucleic acid pool was sequenced and then grouped under different families on the basis of the homology of the primary sequence and the similarity of the secondary structure. Eleven sequences from different families were selected for further characterization via flow cytometry analysis.

Results: Results showed that the sequence ST2P demonstrates affinity for *S.* Typhimurium much more strongly and specifically than other sequences tested. The estimated K_d values of this particularly promising aptamer was 6.33 ± 0.58 nM.

Significance: Our work demonstrates that this aptamer could potentially be used to improve the detection of *S.* Typhimurium.

P3-155 Serotyping of Non-O157 Shiga Toxin-producing *Escherichia coli* by Single Nucleotide Polymorphisms in *gnd*

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) infections may result in serious disease such as hemolytic uremic syndrome, which may lead to kidney failure and death. The most clinically significant STEC serotypes in the US include O157 and six non-O157 serotypes (i.e., O26, O45, O103, O111, O121, O145), which were recently estimated to cause >70% of non-O157 STEC infections in the US. The lack of rapid methods to identify and confirm clinically important non-O157 STEC serotypes may contribute to underreporting of STEC related disease and hinder outbreak investigations.

Purpose: The purpose of this study was to develop a rapid and high-throughput molecular serotyping method to group STEC isolates into seven serotypes (i.e., O157 and the six clinically relevant non-O157 serotypes) by interrogating single nucleotide polymorphisms (SNPs) in *gnd*, which encodes 6-phosphogluconate dehydrogenase.

Methods: A collection of 191 STEC isolates, including isolates belonging to serotypes O103 (n=25), O111 (n=23), O121 (n=24), O145 (n=22), O26 (n=23), O45 (n=22), O157 (n=19), and nine other serotypes (n=33), was assembled and characterized by full sequencing of *gnd* to identify discriminatory SNPs for molecular serotyping. A multiplex SNP genotyping assay was developed to interrogate 11 informative *gnd* SNPs by single base pair extension chemistry and used to characterize the STEC isolate collection assembled here.

Results: Allelic types were assigned to each isolate based on unique combinations of *gnd* SNPs, as determined by the assay, and polymorphisms were confirmed with DNA sequence data. Serotype specific allelic types were identified for each of the seven clinically important STEC serotypes, which allowed the differentiation of clinically important STEC serotypes from non-clinically important STEC serotypes. Three isolates were incorrectly grouped by the SNP assay.

Significance: Molecular serotyping of clinically important STEC isolates by interrogation of 11 SNPs in *gnd* represents an alternative to traditional serotyping for rapid and high-throughput identification and confirmation of STEC serotypes O26, O45, O103, O111, O121, O145, and O157 for surveillance and epidemiological investigations.

P3-156 Evaluation of Commercially Available Loop Mediated Isothermal Amplification (LAMP) Kits for Detection of Foodborne Pathogens

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Introduction: Loop mediated isothermal amplification (LAMP) is a new, powerful innovative gene amplification technique that has emerged as a simple and elegant rapid diagnostic tool for early detection and identification of common foodborne pathogens.

Purpose: The aim of this challenge study was to compare the sensitivity of commercially available LAMP kits (Eiken Chemical Co. Ltd., Tokyo, Japan) in direct comparison to real-time PCR (qPCR).

Methods: Challenge study was conducted by spiking food matrices with serial concentration of pathogen of interest and test for detection using the LAMP kit and qPCR platform (Eiken real-time turbidimetre and Cepheid Smartcycler, respectively). Matrices tested were buffer, ground beef, milk, seeds and sprouts. Test organisms were *Salmonella* species, *L. monocytogenes*, *E. coli* O157:H7, STEC and *C. jejuni*.

Results: The results show that in buffer, the Eiken LAMP kit was a hundred-fold more sensitive in detecting *Salmonella*, ten-fold greater for *E. coli* O157:H7 and *C. jejuni*, identical for Shigatoxin (Stx1 and Stx2) but ten-fold less sensitive for detecting *L. monocytogenes*. The *Salmonella* LAMP kit (having the greatest sensitivity) was further tested in food matrices. Results showed that in ground beef and sprout seeds (alfalfa), LAMP was ten times more sensitive than qPCR and in sprouts (alfalfa), they had identical sensitivity. But in milk, the Eiken LAMP kit was ten thousand-times (4-log) more sensitive.

Significance: The study showed that commercially available Eiken LAMP kit is highly sensitive for detecting *Salmonella* species (especially in milk) but is inferior to qPCR for detecting *L. monocytogenes* and at par or higher for STEC, *C. jejuni*and *E. coli* O157:H7.

P3-157 Rapid Detection of *Brucella* by Loop-mediated Isothermal Amplification

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Introduction: The primary host of *Brucella* spp. is cattle species (*Bos primigenius*) but the bacteria also infect humans and cause zoonotic brucellosis worldwide. Brucellosis causes serious health problems in cattle and humans and economic losses to the cattle industry. Traditional methods for routine detection of *Brucella* is complex and time-consuming, therefore do not fulfill the need of rapid and sensitive detection of this pathogen in milk and other food products. New methods are needed for detecting *Brucella* and providing a reference method for sanitary inspection.

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Purpose: The purpose of the present work was to establish a laboratory Loop-mediated Isothermal Amplification method for rapid detection of *Brucella* spp.

Methods: The Loop-mediated Isothermal Amplification(LAMP) method employs four primers which specifically recognize six distinct sequences of the target DNA and a bst DNA polymerase which has strand displacement activity. The specific LAMP primers of *Brucella* were designed according to a published sequence of gene *omp* 25 using the LAMP primer design support software program. The reaction was carried out at 65°C for 1 h and the products were examined by SYBR Green I stain or by 2.0% agarose gel electrophoresis. The reaction conditions including primers, temperature, time, bst polymerase concentration, dNTPs concentration were optimized. The LAMP reaction was carried out in a 25 μL total reaction mixture volume containing 1.6 μmol/L each of inner primers FIP and BIP, 0.2 μmol/L each of outer primers F3 and B3, 1.6 mmol/L dNTPs, 12.5 mmol/L KCl, 12.5 mmol/L $(NH_4)_2SO_4$, 8.0 mmol/L $MgSO_4$, 0.125% Triton X-100, 0.5 μL of bst DNA polymerase, and 2.0 μL of target DNA. The mixture was incubated at 65°C for 60 min in a heating block and then heated at 80°C for 10 min to terminate the reaction.

Results: The specificity of LAMP method with bsp primers were evaluated by blind amplification of DNA from 1 isolate of *Brucella* and 29 isolates of non-Brucella Gram-negative bacteria. Positive reactions were only observed on the*Brucella* isolate but no other bacteria. The sensitivity of the LAMP method was evaluated by running the reaction on serial diluted bacteria and determined to be 3.81×10^1CFU/mL.

Significance: The LAMP method developed in this work was specific and sensitive and can be potentially applied for rapid detection of *Brucella* spp. in food and environmental matrix.

P3-158 Rapid Confirmation of *Listeria monocytogenes* from Artificially Inoculated Food Matrices Using Partial 16S rDNA Sequence Analysis

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Introduction: The confirmation of *Listeria* to the species level can add up to nine days to the total analysis time when performing a conventional culturing-based isolation starting from a contaminated food product. Nucleic acid sequencing of specific gene targets has the potential to identify *L. monocytogenes* directly from selective/differential media within 8-12 hours, significantly reducing analysis time.

Purpose: A 16S rDNA fragment sequence library was constructed and subsequently used to rapidly confirm *L. monocytogenes* from three types of artificially inoculated food matrices.

Methods: A sequence library was created using multiple foodborne isolates of *L. monocytogenes* serotype 1/2a, 1/2b, 1/2c, and 4b and foodborne isolates of *L. innocua*, *L. welshimeri* and *L. seeligeri*. A 527 bp region between nucleotide positions 5 and 531 from the 5´end of the 16S rDNA gene was amplified and sequenced using the MicroSeq 500ä Bacterial Identification System and an Applied Biosystems 3500xL Genetic Analyzer. Conventional and sequence-based species confirmation was compared using artificially inoculated food matrices.

Results: No intra-species variation was observed for any members of *Listeria* tested. The majority of the inter-species variation within the sequenced region occurred between positions 175 and 200 starting from the 5´ end of the intact 16S rDNA gene of *L. monocytogenes*. *L. monocytogenes* could be distinguished from *L. innocua* by base pair substitutions at positions 180, 197,and 201. *L. monocytogenes* could be distinguished by substitutions at positions 181, 183, 187, 194, 197, 198, 199 and 268 and at positions 176, 181, 182, 183, 196 and 198 from *L. seeligeri* and *L. welshimeri,* respectively. In each case, sequence-based species determination matched traditional biochemical confirmation for artificially inoculated food matrices.

Significance: The use of 16S rDNA sequencing reduced the confirmation and total sample turnaround time for *Listeria* species identification. The ability to confirm the presence of *Listeria* in food products in a shorter time allows regulatory agencies and manufacturers to react sooner to potential human health issues.

P3-159 A Buffer Capacity Model to Determine pH Changes in Acid and Acidified Vegetables Due to Microbial Activity

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Introduction: Acid and acidified food safety depends on maintaining a pH below 4.6 to prevent spore germination. Acids and low pH also inhibit the survival of vegetative pathogens. In addition to lactic and acetic acids in vegetable brines, there are a variety of acidic, basic, and amphoteric compounds that affect buffering and brine pH.

Purpose: Our objective was to develop a buffer capacity (BC) model that could predict pH changes that may affect safety in acid and acidified vegetables due to microbial activity.

Methods: The acid composition of fully, partially and non-fermented (recycled) commercial cucumber fermentation brines was determined by HPLC. The BC of these brines was determined by titration, and compared to the predictions of a preliminary BC model based on the measured acid concentrations. A non-linear optimization algorithm was then used to determine the pKa and concentration of a hypothetical monoprotic buffer that, combined with the known BC, matched the titration data

Results: Comparison of BC data from brines with control solutions containing only lactic acid, acetic acid and NaCl revealed additional buffering was present. The results show that a hypothetical buffer with a pKa of 3.0 and a concentration approximately 20 mM greater than lactic acid of each brine (R^2, 0.98) could predict the observed BC of the brines tested. The hypothetical buffer was presumably a composite of bacterial and plant cell components, including proteins, amino and other acids.

Significance: The BC model can be used for predicting brine pH and pathogen survival in fermenting vegetable brines, as well as the pH changes due to growth of spore forming bacilli in acidified vegetable products. These predictions may be useful for the identification of critical hazards for acid and acidified vegetable products as may be required under the Food Safety Modernization Act.

P3-160 Rapid Molecular Pathotyping of Major *Salmonella enterica* Serotypes Based on Single-nucleotide Polymorphisms (SNPs) in the Adenylate Cyclase (cyaA) Gene

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Introduction: *Salmonella enterica* subsp. *enterica* serotype Enteriditis (*S.* Enteriditis) is the leading cause of salmonellosis worldwide, including the USA. Many *S. enterica* serotypes known to cause foodborne disease are associated with broiler meat contamination. While some serotypes are specific to birds (*S. enterica* serotypes Pullorum, Gallinarum), many represent human zoonotic pathogens (*S. enterica* serotypes Enteriditis, Heidelberg, Typhimurium). The survival capabilities of*S. enterica* serotypes throughout the continuum of poultry production environments (farm to

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processing facilities) is of vital concern to the poultry industry. Environmental parameters that decrease survival of these *Salmonella* populations are important to identify.

Purpose: It is essential to rapidly pathotype the various types of *Salmonella* to determine potential food safety-related issues, especially those that occur within the top 100 serotypes linked to disease and those that have accumulated genetic change that enhances virulence. Serology (O/H antigen testing) and molecular techniques (Premi® Test) have been developed to serotype *S. enterica*, but they are time consuming and rather expensive. More rapid molecular-based methods need to be developed to address the food safety concerns of the poultry industry. The adenylate cyclase gene (*cyaA*) represents an ideal target for these assays due to its necessity in both energy production/metabolism and virulence of *S. enterica*serotypes.

Methods: Primers were designed to target a 300-bp region of the *cyaA* gene, and 5 different SNPs were identified within this region that could distinguish between multiple *S. enterica* serotypes (3 Enteriditis, Typhi, Typhimurim/Heidelberg, and Kentucky). Probes were specifically designed to target these SNPs along the amplified *cyaA* amplicon, and qualitative and quantitative serotyping occurred using a Luminex MagPlex hybridization protocol that we developed and optimized.

Results: The designed hybridization protocol was found to be highly specific to only the target SNP, and thus the target serotype, when testing against an extensive panel of *S. enterica* serotypes. Additionally, the protocol effectively analyzed mixed cultures of several combinations of targeted and non-targeted serotypes. The distribution of these different serotypes, and pathotypes of *S.* Enteriditis, were found to be differentially affected according to the culture conditions under which the strains were grown, indicating potential biases in these cultural conditions towards certain serotypes/pathotypes.

Significance: These data contribute to a greater understanding of not only the survival of *S. enterica* serotypes under different cultural conditions, but also the survival dynamics of the separate serotype and virulence types relative to one another. Expanding this method to include other serotype-specific SNPs within the *cyaA* gene, or applying it to environmental poultry production samples will allow the poultry industry to not only rapidly determine how much *Salmonella* is present, but more importantly which serotypes are present, but also which environmental parameters control the survival of the food safety related serotypes.

P3-161 *Withdrawn*

P3-162 Selection of DNA Aptamers with Binding Affinity to Human Norovirus

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Introduction: Human noroviruses (HuNoV) are the leading cause of foodborne disease. Currently, there is no easy way to detect HuNoV contamination. A major reason for this is the so-called "needle in a haystack" dilemma, meaning that it is necessary to concentrate and purify small numbers of viruses from the sample matrix prior to the application of molecular-based detection. Aptamers [small, single-stranded (ss) DNA or RNA molecules that naturally fold into complex three-dimensional shapes] are emerging ligands for pathogen capture. These demonstrate advantages over traditional capture ligands like antibodies, including reduced cost, ease of production and modification, and improved stability.

Purpose: To develop and characterize ssDNA aptamers with binding specificity to the HuNoV GII.2 strain Snow Mountain virus (SMV).

Methods: Aptamers were selected from a large combinatorial library of random ssDNA molecules using the SELEX (Systematic Evolution of Ligands by Selective Enrichment) method targeting purified, intact virus. After multiple rounds of SELEX (and counter-selection), enriched aptamer pools were cloned, sequenced and their secondary structure analyzed using DNA Mfold. Preliminary data on their binding affinity was determined using an Enzyme-Linked Aptamer (ELA) assay developed as part of this work.

Results: Thirty-eight aptamer candidates were identified. Of these, five aptamer sequences (SMV-5, SMV-18, SMV-22, SMV-21S9, SMV-22S9) were most predominant, represented from 2-5 times in each purified aptamer pool. Sequence analysis of the aptamers revealed only a few common domains but similarity in predicted structural folding, with all aptamers having a dominant loop and three protruding hairpins. Equilibrium dissociation constants (K_d), a measure of binding affinity, were 0.04, 0.045, 0.142, 0.05, 0.016, μM for aptamers SMV-5, SMV-18, SM-22, SMV-21S9, and SMV-22S9, respectively.

Significance: Aptamers with binding affinity to SMV are promising ligands for pathogen capture prior to molecular detection. Future studies focus on using these to develop practical, inexpensive approaches to concentrate HuNoV from foods and environmental samples.

P3-163 Investigating the Viability and Culturability of *Escherichia coli* in a Novel Model Orange Juice Using Flow Cytometric Techniques

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Developing Scientist Competitor

Introduction: Highly acid resistant *Escherichia coli* O157:H7 could survive the low pH of orange juice due to its ability to adapt to acidic conditions. It is also known that natural stress factors commonly encountered by bacteria during mild food production such as heat and cold can induce a "viable but non-culturable" (VBNC) state in *E. coli*. Flow cytometry (FCM) can be used to monitor changes in cellular viability and morphology, both in cells that are able to grow and in VBNC cells.

Purpose: The aims of this study were to investigate the viability and culturability of *E. coli* subjected to a novel Model Orange Juice (MOJ) using FCM and plate counting.

Methods: *E. coli* K-12 MG1665 in different growth phases (OD_{650} = 0.5, 0.75 and 1) were added to a MOJ (pH = 3.2, containing sucrose, fructose, glucose, citric and malic acids and potassium citrate). The behavior of *E. coli* in MOJ was investigated during 24 hours storage (4 °C and 37 °C) using traditional (recovery on nutrient agar plates) and FCM (propidium iodide/bis-oxonol viability staining) techniques. The results were statistically analyzed using the Mann-Whitney test.

Results: FCM results showed a significant decrease ($P < 0.001$) in the number of viable cells for each sample after three hours of incubation, followed by a modest increase ($P < 0.05$), regardless of the incubation temperature and/or growth stage. For instance, for mid-log phase cells (OD_{650} = 0.5) at 4 °C, there was a 1.12 ± 0.04 log decrease and a 0.30 ± 0.07 log increase in cell count after 3 and 24 hours, respectively. Furthermore, compared to FCM, plate counting identified significantly fewer viable cells (4.26 ± 1.06 log, $P < 0.001$) after 24 hours, probably due to an increase in the proportion of VBNC cells.

Significance: These data suggest that plate counting grossly underestimated viable cells despite using the most common resuscitation and recovery technique. Considering the very low dose of infection of *E. coli* O157:H7, FCM could be a powerful technique for its identification in foods.

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P3-164 Selection and Characterization of DNA Aptamers with Binding Specificity for *Listeria* spp. and the Use of DNA Aptamers for Capture of *Listeria* spp. Prior to the Application of qPCR for Detection

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Introduction: More rapid detection of foodborne pathogens could be achieved if the target(s) were concentrated and purified from the sample prior to detection by quantitative PCR (qPCR). Aptamers [small, single-stranded (ss) DNA or RNA molecules with target binding affinity] are emerging ligands to facilitate pathogen capture. These demonstrate advantages over traditional capture ligands like antibodies, including reduced cost, ease of production, and high stability.

Purpose: To identify ssDNA aptamers with binding specificity to *Listeria monocytogenes* and use these for capture and subsequent qPCR detection of the organism.

Methods: For aptamer selection, SELEX (Systematic Evolution of Ligands by EXponential enrichment) was applied to a biotin-labeled ssDNA combinatorial library. After multiple rounds of selection and counter-selection, aptamers with binding affinity to *L. monocytogenes* were separated, sequenced, and characterized with the aid of flow cytometry. One aptamer (Lbi-17) was conjugated to streptavidin-coated magnetic particles and used for capture and subsequent detection of *L. monocytogenes* using qPCR targeting the *hly* gene.

Results: Five aptamer candidates were identified, all having binding affinities of 18-24% as evaluated by flow cytometry. Although selected for using *L. monocytogenes*, these aptamers showed similar binding affinity for other members of the*Listeria* genus (13-21%), and low binding affinity for non-*Listeria* species. Aptamer Lbi-17 was chosen for further characterization based on its high binding affinity for *Listeria* and relatively low binding with non-*Listeria* spp. The dissociation constant (K_d) of Lbi-17 was 35.7 ± 8.0 μM. When Lbi-17 was conjugated to magnetic beads and used for capture and detection of *L. monocytogenes*, capture efficiency (CE) ranged from 15-19%. A similar CE was observed for IMS-qPCR (13-17%), but the limit of detection for the aptamer-based method was one log lower (better) at 60 CFU/ml.

Significance: This study shows proof-of-concept that ssDNA aptamers can be used for capture and detection of a common foodborne pathogen, with detection limits better than those observed for IMS-qPCR.

P3-165 Development and Validation of a Test System to Detect *Brucella abortus* in Whole Milk, Soft Cheese and Leafy Greens

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Introduction: Brucellosis is a zoonotic disease that affects both humans and animals. In the United States, brucellosis in humans frequently occurs after ingestion of unpasteurized milk or dairy products, or after being in contact with infected meat or placenta of infected animals. The causative agents, *Brucella* spp., are fastidious, slow-growing organisms that do not grow in conventional laboratory media.

Purpose: To develop a test system for the detection of *Brucella* spp. from food matrices, and to validate its performance on artificially-inoculated whole milk, soft cheese and leafy greens.

Methods: *Brucella abortus* S19 (vaccine strain) was artificially inoculated into home-made soft cheese (25 g), leafy greens (25 g) or whole milk (25 ml), stored overnight at 4 °C, and enriched with Brucella media supplemented with polymyxin B, cycloheximide, vancomycin, and nalidixic acid. Twenty of 25 samples for each food matrix were inoculated with 1 to 80 CFU, and 5 served as uninoculated controls. Sample aliquots were taken daily for 5 days and analyzed using a test system that included multiplex PCR assay, lateral flow immunoassay (LFI) and immunomagnetic particles (IMP).

Results: A test system for screening and confirming the presence of *Brucella* in food samples was developed. The test system was validated in house using artificially inoculated whole milk, soft cheese and leafy greens at a level of 1 to 80 CFU/25 g or ml of food matrices. Using a combination of IMP-multiplex PCR, *B. abortus* S19 was detected in food samples as early as after 2 days of enrichment, and all confirmed *Brucella* -positive samples were identified when incubation was extended up to 3 to 4 days. LFI detected all PCR-positive samples after 4-days of enrichment. The PCR-negative and uninoculated enrichment samples did not produce any positive signal from IMP-multiplex PCR and LFI even after 7 days of enrichment. These results suggest that the multiplex PCR and LFI methods exhibited 100% sensitivity (ability to detect positives when positives are present) and 100% specificity (ability to declare a negative when sample is truly negative). The limit of analytical sensitivity of the multiplex PCR assay was 10^4 CFU/ml, while that of the LFI was 10^5 CFU/ml, which explains the additional 24 hr enrichment required to detect positive signals using LFI from samples that had already tested positive using multiplex PCR.

Significance: This test system provides a rapid and robust method for detection and isolation of *Brucella* spp. from some contaminated foods.

P3-166 Dye-incorporated Chitosan-based CO_2 Indicator to Monitor Food Freshness

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Introduction: Quality and freshness indicators that are placed inside sealed food packages give consumers an easier way to find microbial spoilage of food. As the microbial growth and subsequent spoilage leads to production and accumulation of carbon dioxide (CO_2), the partial pressure of CO_2 in headspace of food package can be considered as a quality indicator of food.

Purpose: The aim of this study is to develop a chitosan-based CO_2 indicator which exhibits prominennt visual color change depending on different CO_2 levels. With the increase of CO_2 levels in the headspace of the package, chitosan suspension develops transparency due to its dissolution at lowered pH.

Methods: For fabricating a CO_2 indicator, 0.3% brilliant blue R-250 dye was incorporated into the chitosan dissolved in 0.1 M HCl aqueous solution. The pH of the chitosan-dye mixture was adjusted to 7 and stirred for 2 h. The CO_2indicator was stored in an incubator filled with CO_2 gas to investigate the time-dependent visual changes. Transparency level and color of the CO_2 indicator solution were measured every 20 minutes.

Results: At the start of incubation, the indicator solution was opaque because chitosan is insoluble at neutral condition (pH 7). Upon increase of CO_2 levels, the pH of indicator solution decreased due to carbonic acid formation; thereby, the solution became transparent and color changed to dark blue. The signal response of the CO_2 indicator was time dependent; pH of the CO_2 indicator was decreased below pH 6 under 100% CO_2 incubation. In addition, under this condition, transparency and blueness of the CO_2 indicator increased over time.

Significance: The visual chitosan-based CO_2 indicator can indicate partial pressure changes of CO_2 and has a potential to be applied to examine optimal ripeness of fermented vegetables such as kimchi and microbial spoilage.

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P3-167 Growth and Repair of *Escherichia coli* Non-O157:H7 STEC Strains in Selective Enrichment Broths

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a group of pathogenic *E. coli* strains characterized by O157:H7 and non-O157 serogroups. The non-O157 infections have recently emerged as a significant concern in various countries. Within the *E. coli* non-O157 group, the Centers for Disease Control has identified six serogroups (O26, O111, O45, O121, O103 and O145) that are responsible for over 70% of the non-O157 STEC-associated illnesses. The resistance of these six serogroups to selective ingredients can be quite varied. Therefore, a selective enrichment broth must take this into account in order to repair and rapidly grow non-O157 cells with inhibition of its competing organisms.

Purpose: To develop and assess R & F® non-O157 STEC selective enrichment broth (STEC-SEB).

Methods: All bacterial strains were grown in brain heart infusion broth at 35 °C for 24-48 h. An isolate from each of the six non-O157 STEC serogroups were freeze injured in 10% skim milk and stored at -76 °C. Repair of injured cells were ascertained using recovery of cells on brain heart infusion agar versus MacConkey agar containing 0.6% bile salts #3 agar. Growth and/or repair of the isolates were evaluated in the USDA recommended modified tryptone soy broth (mTSB) with 8 mg/l of novobiocin and STEC-SEB compared with tryptic soy broth (TSB) incubated at 42 °C.

Results: Repair of injured non-O157 STEC strains were similar in TSB and STEC-SEB, whereas, resuscitation of injured cells was delayed in mTSB. Also, mTSB inhibited the growth of 10% of the non-O157 STEC (includes 28 isolates) strains tested, whereas, STEC-SEB grew all the tested non-O157 STEC strains similar to TSB. Selectivity of competing bacterial strains in mTSB and STEC-SEB was similar.

Significance: With the growth of all the tested non-O157 STEC strains to a titer of 10^8/ml after 24 h at 42 °C coupled with a limited selectivity, the use of STEC-SEB will result in fewer false-negative isolations compared with the USDA recommended mTSB.

P3-168 Development of an Extraction and Concentration Procedure for the Detection of Enteric Viruses in Soil

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Introduction: Enteric viruses, including Norovirus, rotavirus and hepatitis A viruses have been recognized as being the leading cause of non-bacterial gastroenteritis and infectious hepatitis outbreaks around the world. Understanding the prevalence and persistence of enteric viruses in environmental samples is an important issue for the effective control of these infections. Commercial extraction kits developed for soil allow the treatment of very small volumes (0.5 g – 5 g) and viruses are generally in low concentration in the environment.

Purpose: The aim of this study was to develop an efficient extraction and concentration procedure for enteric viruses from larger samples.

Methods: 25 g of soil (black earth, loamy and sandy soils) were spiked at various concentrations between 10^5 and 10^2 PFU/g of murine norovirus (MNV) and feline calicivirus (FCV) used as model for enteric viruses. The soil samples were mixed with different buffers, additives (PVPP, activated carbon) and concentrated on ultra-filtration units. Viral genetic material was extracted by different commercial kits and conventional RT-PCR and Taqman real-time RT-PCR systems were used for detection.

Results: Different combinations of buffers, additives, extraction kits were tested and the more efficient procedure to remove PCR inhibitors was composed of MEM buffer, PVPP and the Dynal beads extraction. This procedure has allowed the detection of 10^2 PFU/g of MNV and 10^3 PFU/g of FCV in the three types of soil samples.

Significance: This method allows the concentration of MNV and FCV viral particles from 25 g of three different kinds of soil frequently used for fresh products culture. This method can be applied for the detection by molecular methods of other unculturable viral species, such as Norovirus, responsible of foodborne diseases, and to increase our knowledge on their persistence in soil and its impact on viral contamination of fresh horticultural products.

P3-169 Gluten Detection with a New Generation of Monoclonal Antibody

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Introduction: Gluten is the main group of proteins in grains such as wheat, rye, barley and, to a lesser extent, oat. Celiac disease is an immune-mediated enteropathy caused by the ingestion of gluten. Recent discussions about celiac disease have moved from the concept of gluten detection to detection of the cereal protein fractions that are toxic to persons intolerant to gluten; an approach which is closer to the provisions of the Codex Standard 118:1979.

Purpose: In this work the monoclonal antibody G12, raised against the QPQLPY peptide from a toxic fragment called 33-mer of the gliadin, was used to develop a sandwich enzyme linked immunosorbant assay (ELISA), AgraQuant®Gluten G12 and a lateral flow device (LFD), AgraStrip® Gluten G12.

Methods: Studies were performed on the antibody's specificity, as well as the limit of detection of the test kits. Common and problematic food matrices, rinse water and swabbing from steel and plastic surfaces were evaluated.

Results: The limit of detection (LOD) of the ELISA test was determined to be 2 ppm gluten, with a quantitation range of 4 to 200 ppm. Spiked samples and processed food samples showed comparable performance with a Mendez R5 ELISA method. The LOD of the lateral flow device was determined to be 5 ppm gluten in spiked commodities. By varying the amount of dilution buffer, it was possible to adjust to cutoff levels of 5, 10 and 20 ppm. When testing rinse water, variation in pH from 5 to 9 did not affect the results.

Significance: During validation of these tests, positive and negative responses to oat varieties were obtained. However, the positive results appear to be a specific reaction of the antibody with the toxic fragment, rather than a non-specific positive signal. This suggests that the G12 antibody may shed light on the debate concerning potential immunotoxicity of oats.

P3-170 Production of Monoclonal Antibodies against Intimin-γ and Intimin-α

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Introduction: Intimin, a 97-KDa outer membrane protein, is one of the virulence factors of enterohaemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC). The detection of EHEC from foods relies on the effective recognition of intimin by using a molecular approach.

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Purpose: To generate monoclonal antibodies against intimin-γ (intG) and intimin-α (intA) for detecting EHEC.

Methods: The gene encoding the N-terminal variable region of the intG from *E. coli* O157:H7 and intA of IEH EPEC strain 8111 were PCR-amplified, cloned and expressed. The gene products were purified and the recombinant intG and intA were used to prepare monoclonal antibodies (mAb) which were characterized using ELISA, Western blot and immunofluorescent microscopy. The anti-intA was used to prepare immunomagnetic particles (IMP).

Results: The recombinant N-terminal region of intG and intA were used to generate anti-intG 5F11 and anti-intA 20C6 mAb. mAb 5F11 recognized the recombinant intG and the 97-KDa protein from *E. coli* O157:H7 lysate, while mAb 20C6 recognized only those of the EPEC strain 8111. Immunofluorescent microscopy using FITC-labeled mAb suggested that 5F11 and 20C6 attached to the surface of *E. coli* O157:H7 and EPEC strain 8111, respectively. mAb 20C6-IMP was prepared, and used to concentrate EPEC strain 8111 grown in Dulbecco's modified Eagle's medium (DMEM). Approximately 20% of the cells were recovered after treatment with 20C6-IMP.

Significance: The monoclonal antibodies recognized both the recombinant and native intimins from cell lysates of either *E. coli* O157:H7 or EPEC strain 8111, and could be used for detecting these isolates. The anti intimin mAb can be attached to IMP and used to concentrate intimin-producing EHEC and EPEC.

P3-171 Development and Validation of a Semi-quantitative Lateral Flow Device for Aflatoxin Detection in Corn and Nuts

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Introduction: Aflatoxins are recognized as the most important group of mycotoxins. As secondary metabolites, they are synthesized by *Aspergillus* species (*A. flavus* and *A. parasiticus*) on agricultural produce grown under stressful conditions. The mold is found in soil and on damaged or decaying grains, fruits or nuts. There are several different types of aflatoxins, but B_1, B_2, G_1 and G_2 are the most common. Aflatoxin B_1 is considered the most toxic and carcinogenic substance produced in nature. FDA has established action levels for the amount of aflatoxins allowed in food or feed to protect human and animal health. The lack of availability and simplicity of routine qualitative and semi-quantitative aflatoxin detection at storage/sorting facilities remains an essential problem in controlling aflatoxin contamination.

Purpose: To develop a semi-quantitative method for aflatoxin detection in different food matrices that is rapid, simple to use and does not require expensive equipment.

Methods: A lateral flow device was developed for semi-quantitative aflatoxin detection based on a competitive inhibition immuno-assay. For this purpose, high-affinity monoclonal antibodies against aflatoxin B_1 were obtained, conjugated to colloidal gold particles and used as a detection reagent in a lateral flow device. For the capture reagent, aflatoxin-BSA was immobilized on a nitrocellulose membrane. To achieve semi-quantitative aflatoxin detection, different amounts of aflatoxin B_1-BSA were applied on several test lines. For the procedural control, goat anti-mouse IgG antibodies were immobilized downstream from test lines on the lateral flow device.

Results: **:** A lateral flow test device was developed that allows users to determine aflatoxin concentration in a single step. Levels of 5 ppb, 20 ppb and 100 ppb (B_1+B_2+G_1+G_2) are used as cut off levels. To validate the lateral flow method, 6 corn samples and 25 peanut samples spiked with different aflatoxin concentrations were tested and the results were compared with the gold standard method of quantitative HPLC.

Significance: A semi-quantitative method for aflatoxin detection was developed that allows, in a single step, determination aflatoxin levels at low (less then 5 ppb), middle (less then 20 ppb, but more than 5 ppb) or high range (more the 100 ppb) of contamination.

P3-172 Efficacy of Interventions for Reducing *Salmonella* on Raw Turkey Parts Used in Ground Turkey Production

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Introduction: *Salmonella* is a Gram-negative bacterial pathogen present in raw poultry. Specific serotype and antibiotic resistant *Salmonella* have been associated with recent outbreaks linked to the consumption of ground turkey.

Purpose: The goal of this study was to determine efficacy of multiple concentrations and contact times of two interventions: Peracetic Acid (PAA) or Cetylpyridinium Chloride (CPC) against *Salmonella* on raw turkey parts used in production of ground turkey.

Methods: A five-strain *Salmonella* cocktail using separate high level (5-6 log) and low level (0.5-1 log) inoculums were applied to parts. High level inoculated parts were dipped into solutions of PAA (200, 500, 1000, and 2000 ppm) or CPC (0.4% and 0.6%). All treated and untreated whole parts were sampled using the USDA-FSIS poultry parts rinse method. Based on results from the high level inoculum, the low level inoculation study was initiated to confirm effectiveness through grinding. Treated and untreated parts were sampled prior to grinding. Low level inoculated parts were dipped into solutions of PAA (1000 ppm) or CPC (0.2% and 0.4%). Immediately after grinding, 25-gram and 375-gram samples of product were collected for testing.

Results: *Salmonella* quantitative results from high inoculated parts dipped in PAA at 1000 ppm at a minimum for 10 seconds generated greater than a 1-log reduction. CPC at 0.4% and 0.6% at a minimum of 10 seconds generated greater than 2 logs reduction. *Salmonella* was reduced quantitatively and qualitatively to undetectable levels from low level inoculated treated parts sampled prior to and after grinding.

Significance: PAA and CPC are effective interventions for reducing the presence of *Salmonella* in ground turkey.

P3-173 Foam/Vacuum Extraction, Hollow Fiber Concentration and Quantitative PCR for Detection of *Salmonella* on Model Food Processing Surfaces

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Introduction: Effective pathogen detection is crucial to maintaining the safety of our food supply. While much effort has been aimed at improving the final detection step, pre-analytical sample preparation remains an underexplored aspect of pathogen testing. Special challenges to detection may occur when pathogens are present at low concentrations, and/or distributed over large surface areas.

Purpose: To evaluate the use of a combined surface extraction and filtration approach for removal and concentration of *Salmonella* from artificially contaminated stainless steel surfaces prior to molecular detection; to compare this approach with traditional swab-based sampling methods.

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Methods: *Salmonella* Typhimurium ATCC 14028 was diluted in PBS and a total of 10^6 CFU were distributed evenly over the surface of small (4" x 4") or large (24" x 4") stainless steel plates (type 304, #4 finish). After drying, contaminated surfaces were sampled using three methods: cotton swab, rayon swab and foam/vacuum surface extraction plus hollow fiber concentration (E+C). *Salmonella* recovery with each method was assayed by quantitative PCR (qPCR) targeting the *invA* gene.

Results: For small plates, use of the E+C method led to a 10-fold increase in recovery of *S.* Typhimurium over the swab-based methods. Sampling efficiency was higher for the larger plates, where the E+C method yielded a 100-fold increase in recovery of *S.* Typhimurium over the swabs. Lower recovery from the smaller plates was attributed to difficulties in maintaining a vacuum seal with the sample surface, as the dimensions of these plates were similar to those of the extractor sampling head.

Significance: Combined use of foam-based vacuum extraction and subsequent hollow fiber concentration enabled enhanced recovery of *S.* Typhimurium from a model food processing surface compared to traditional swab-based methods, allowing concentration of contaminant cells from bulk surfaces into small sample volumes compatible with molecular testing methods such as qPCR.

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