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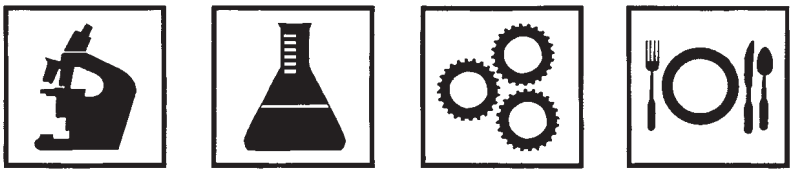

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ABSTRACTS

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IVAN PARKIN LECTURE ABSTRACT

20 Years Later, Where Were We, Where are We and Where are We Going?

William Marler
Marler Clark, LLP PS
Seattle, Washington

Many would say that the 1992–1993 Jack in the Box *E. coli* O157:H7 outbreak was the beef and restaurant industries' "911." William "Bill" Marler was a young attorney in Seattle in the right place at a wrong time – right for an aggressive litigator, but wrong for the over 600 sickened, at least 50 with acute kidney failure and four deaths – all from doing the all American thing – eating a hamburger!

Mr. Marler is proud to give the Ivan Parkin Lecture in honor of those who were sickened and those who died. However, after over 20 years of being involved in every major foodborne illness outbreak that has occurred in the United States, Mr. Marler recognizes the major breakthroughs that have occurred in academics, government, but, most importantly, industry, in trying to make our food supply safer.

Mr. Marler will walk through the history of the Jack in the Box case and how it led to changes in how everyone viewed food safety. Although safer food has always been the goal, getting agreement on who is responsible and how to accomplish it has been a struggle.

In the end, however, professionals who showed a commitment to science and their craft have led a revolution to a safer food supply. Foodborne illness cases are down, outbreaks are down, but the commitment to make food safer remains the goal.

As illnesses and outbreaks fade, the challenge is to keep all – from farm to fork – focused on doing their jobs and not resting on the improving statistics.

JOHN H. SILLIKER LECTURE ABSTRACT

Bringing Science-based Risk Analysis to Practice to Further Improve Food Safety

Leon Gorris
Unilever
Shanghai, China

In today's world, food products and food ingredients are traded among many different countries and often sourced from or sold to geographical regions far away from their country of origin. Such a globalized food system presents the modern business model and serves consumer interests, increasing the overall availability and access to a variety of foods. Evidently, as food supply chains become increasingly longer and more complex, the challenge for governmental food safety control and private food safety management may increase too. Some high level food safety incidents with regional or even global impacts serve as stark examples of the consequences of food safety assurance fails somewhere in a globalized system.

Ensuring food safety in practice

As the primary suppliers of food to the wider public, the food industry at large has an important role in food safety assurance. At a basic but very effective level, application of best practices such as GMP and GHP, in conjunction with suitable systems to manage individual food processing operations, such as those based on the principles of HACCP, will, to a large extent, help food industry to effectively manage potential food safety hazards during normal operations as well as in unexpected situations. To further strengthen food safety management, food industry of all sizes would, first and foremost, adequately and consistently implement those best practices and management systems that have time and again proven its usefulness. In the globalized food system food supply chain there are indeed challenges to adequate food safety management in every day practice. But there are also opportunities for future strengthening and harmonization, such as making use of the good resources and guidance available from global food safety organizations and to align more closely to modern approaches to food safety assurance adopted by Codex Alimentarius. This modern approach is framed as Risk Analysis, a science-based paradigm that focused on risks for effective food safety assurance, as opposed to more traditional, hazard-based, approaches.

Application of Risk Analysis to further improve food safety

Application of the concept of Risk Analysis for food safety is advocated by international organizations such as the WHO and FAO. Risk analysis integrates science-based risk assessment and risk communication with risk management. Risk assessment evaluates the risk posed by particular food safety hazards in order to provide options to risk managers in choosing actions that are proportional and effective in controlling food safety. Risk communication provides for an interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions, among all stakeholders: risk assessors, risk managers, consumers, industry, the academic community and other interested parties. More and more competent authorities around the world have embraced Risk Analysis principles as the international gold standard for food safety decision-making related to public health protection and international trade. It allows increasingly consistent local and international decision-making and focuses resources on the priority issues. From an industry's point of view, aligning food safety management with outcomes of risk-based governmental decisions is extremely relevant and has many advantages. It can help guide the adoption of appropriate control measures in relation to the level of risk from possible food safety hazards, thus ensuring protection for consumers. It is not necessary for the food industry at large to adopt risk analysis for food safety management in every day practice as their role is to execute control measures properly through GHP/GMP and HACCP. However, some food companies have successfully applied risk analysis principles in designing food products and food processing methods before food products are brought to market.

Symposium Abstracts

MERS-CoV-An Emerging Pathogen Potentially Linked to Food Production

PETER BEN EMBAREK: *World Health Organization, Geneva, Switzerland*
ARON HALL: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Middle East Respiratory Syndrome (MERS) is a viral respiratory disease caused by a novel coronavirus (MERS-CoV) that was first identified in Saudi Arabia in 2012. As of mid-June 2014, 700 laboratory-confirmed cases of human infection with MERS-CoV have been reported to WHO, including at least 209 deaths. To date, 22 countries have reported cases but so far all cases have been exposed in the Middle East. The virus has been isolated from dromedary camels and seroprevalence studies seems to indicate it is circulating widely in the camel population of the Arabian Peninsula ad parts of Africa. It is not yet understood exactly how people become infected but direct or indirect contact with camels is suspected. Camels are believed to be a likely primary reservoir for MERS-CoV. Others cases have acquired their infection through human to human transmission especially in health-care associated outbreaks. However, the virus does not seem to be easily transmissible among people. The virus has been detected in camels at slaughtering and a number of slaughterhouse workers and farm workers handling camels are seropositive for MERS-CoV indicating a possible higher risk of contracting the infection along that food chain. Trace of MERS-CoV have also been detected in camel milk and this has prompted fear that consumption of camel milk and other raw camel products could be source of infection. The session will present an update on our understanding of this emerging disease and its possible links to food production and food consumption.

S1 The Rise of the Genomes – How Whole Genome Sequencing Will Transform Food Safety

KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*
BRUNO SOBRAL: *Nestle, Lausanne, Switzerland*
JORGEN SCHLUNDT: *The Danish Food Institute, Soborg, Denmark*
PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ERIC BROWN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*

Whole genome sequencing is an emerging technology that is increasingly being used by academia, clinical, public health and food regulatory agency laboratories. It is now possible to sequence the genome of a bacterial strain in approximately one day at a cost of about $100 and the speed is increasing as the cost is going down. The technology is attractive because it may be used to identify, characterize and subtype microbes with precision like never before. It is beginning to enter food laboratories, too. However, it is an emerging technology: sequencing platforms are constantly evolving as is the software that is used to analyze the data and interpretation of the data is rarely straight forward. In this symposium, the basics of the technology will be explained in layman terms, as will the analytical tools and how the technology could be beneficial to the food industry. Implementing a technology as complex as whole genome sequencing is a monumental task and if we want to be able to compare data between laboratories globally, a number of technical, logistical, political and ethical questions needs to be addressed and agreed upon internationally; these aspects will be dealt with by the so-called Global Microbial Identifier initiative. Finally, the public health and food regulatory experience with the technology will be presented as will how it is anticipated the technology will be used in food regulation.

S3 Mechanically-tenderized Meats – Should We be Concerned? Current Status and Future Directions

JEFFREY FARBER: *Health Canada, Ottawa, ON, Canada*
XIANQIN YANG: *Agriculture and Agri-Food Canada, Lacombe, AB, Canada*
WILLIAM SHAW: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
JOHN LUCHANSKY: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
BETSY BOOREN: *American Meat Institute Foundation, Washington, D.C., USA*
JAMES REAGAN: *National Cattlemen's Beef Association, Centennial, CO, USA*
CHRISTOPHER WALDROP: *Consumer Federation of America, Washington, D.C., USA*

Mechanically-tenderized meats (MTB) describe those non-intact beef products, i.e., steaks, roasts, chops, etc., where the meat surface is penetrated by blades or needles and this manipulation is not detectable by the naked eye.

Some meat handlers and even consumers at home tenderize cuts of beef, including steaks and roasts, using machines or tools made for this process. Mechanically tenderizing meat is a very common practice and has been used by suppliers, restaurants and retailers for many years to improve the tenderness and flavour of cooked beef. However, this can present a risk to the consumer if the surface of the meat is contaminated with bacterial pathogens and these spread from the surface to the inside of the meat when the meat is being mechanically-tenderized.

Experts on MTB research from academia, government and industry will present recent research, risk assessment and policy findings concerning those factors that are important in the safety of MTB, as well as discussing the various risk management strategies that can be put in place to manage the potential risks.

S4 Quantitative Aspects of Detection Methods for Food Safety Sampling

HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands*
RABEB MILED: *ANSES, Maison-Alfort, France*
PATRICE ARBAULT: *BioAdvantage Consulting, Orlienas, France*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*
J. STAN BAILEY: *bioMérieux, Durham, NC, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*

Sampling and testing of foods for the presence of pathogens is important to verify acceptable food safety levels. Analytical testing methods often incorporate an enrichment procedure to resuscitate and amplify the target pathogen to higher concentrations allowing subsequent detection using classical or rapid molecular detection methods. These enrichments and detection methods are not perfectly selective and sensitive. This symposium

focuses on quantitative and ecological aspects of this detection process and its relevance for food safety management by focusing on (1) the quantitative ecology of the target organism and background flora during non-selective and selective enrichment (2) advances in levels of detection, sensitivity and selectivity of different detection methods (broad range of classical and rapid methods). (3) the effects of sensitivity and selectivity on the performance of sampling plans. This symposium will address these issues with leading experts in the field, discussing classical and rapid detection methods, enrichment ecology, their performances and relevance for food safety management.

S5 Ranking More Than Risk: Multicriteria Approaches to the Prioritization of Foodborne and Zoonotic Pathogens

ARIE HAVELAAR: *Centre for Infectious Disease Control, RIVM, Bilthoven, Netherlands*
YUHUAN CHEN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
MICHAEL BATZ: *University of Florida, Gainesville, FL, USA*
VALERIE DAVIDSON: *University of Guelph, Guelph, ON, Canada*
VICTORIA BROOKES: *University of Sydney, Sydney, Australia*
VICTOR DEL RIO VILAS: *Pan American Health Organization (PAHO), Rio de Janeiro, Brazil*

A risk-based approach to setting priorities and developing management strategies for foodborne and zoonotic pathogens often involves the qualitative or quantitative ranking of hazards or potential control measures. These rankings may be based on estimates of incidence or measures of disease burden such as disability-adjusted life years (DALYs), but there is a growing recognition that infectious diseases have numerous dimensions beyond these metrics. A number of multi-factorial approaches have been developed across the world to rank pathogens not only on their direct impacts to public health, but also on aspects such as distributional factors, epidemic potential, disease trends, socioeconomic factors, impacts on trade or domestic industries, availability or feasibility of control measures, impacts on ecological or wildlife, and consumer preferences, among others. There is a range of novel approaches that have been developed, as the attributes of importance, quality of scientific data, and availability of expertise may vary by country or depend on aspects of the risk management context.

This symposium will include presentations on a number of multicriteria frameworks that have been developed and applied to address food safety, zoonotic disease, and animal health in a number of different countries. The symposium will focus on consideration of the appropriate metrics for different risk criteria and methods for aggregating evidence across multiple criteria. Presenters will address how these multicriteria approaches addressed needs of the decision making process and ultimately how the results were used by policymakers. This symposium will introduce IAFP attendees to an important methodology for prioritization that is being increasingly used throughout the world, but which has not had wide coverage at IAFP in the past.

S6 *Cyclospora*: Recent Foodborne Outbreaks and Challenges

REBECCA HALL: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
JULIE HARRIS: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, USA*
SOCRATES TRUJILLO: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
PALMER ORLANDI: *U.S. Food and Drug Administration, Rockville, MD, USA*
SEAN PICQUELLE: *Taco Bell Corp., Irvine, CA, USA*

Cyclospora cayetanensis infections associated with fresh produce have emerged as a serious public health concern in the U.S. in the past decade. In 1996 and 1997, cases of infection in the US and Canada were associated with consumption of berries imported from Guatemala. Outbreaks of cyclosporiasis were linked to basil in 1997, 1999, 2001, and 2005 and to snow peas in 2004 and 2009. In 2013, more than 600 cases of cyclosporiasis potentially linked to fresh produce were reported in 23 states. These outbreaks reflect the complex nature of *C. cayetanensis* and its associations with fresh fruits and vegetables. Difficulties in epidemiologic investigations and confirmation of sources of *C. cayetanensis* responsible for outbreaks have been due in part to the unavailability of sensitive analytical tests for its detection. Epidemiologists and food parasitologists from CDC, FDA, and academia will address the biological properties of *C. cayetanensis* in this symposium. Recent advances in the biology, control, detection, and prevention will be presented. In addition, this symposium will describe the challenges and limitations in outbreak investigations, particularly the one in 2013. A representative from the food industry will address preventive measures implemented to minimize the risk of outbreaks associated with fresh produce.

S7 Update on the Shiga Toxin-producing *Escherichia coli* Coordinated Agricultural Project (STEC-CAP)

RODNEY MOXLEY: *University of Nebraska-Lincoln, Lincoln, NE, USA*
DAVID RENTER: *Kansas State University, Manhattan, KS, USA*
JOHN LUCHANSKY: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
PIUS EKONG: *Kansas State University, Manhattan, KS, USA*
MICHAEL SANDERSON: *Kansas State University, Manhattan, KS, USA*
DANIEL GALLAGHER: *Virginia Tech, Blacksburg, VA, USA*
GARY ACUFF: *Texas A&M University, College Station, TX, USA*

This symposium will provide a progress update on the Shiga toxin-producing *Escherichia coli* (STEC) Coordinated Agricultural Project (CAP). The STEC-CAP is supported by a $25 million grant coordinated through USDA's Agriculture and Food Research Initiative (AFRI) and administered through USDA's National Institute of Food and Agriculture (NIFA). The STEC-CAP effort is supported by a multi-institutional, multi-disciplinary team of land-grant universities and government agencies led by the University of Nebraska–Lincoln. The team is conducting research and educating on how STEC contamination and outbreaks occur and spread throughout the beef production/processing chain, and on how science and technology can best be used to mitigate STEC risks. New and enhanced knowledge from this work will yield practical, effective information and communication tools to reduce STEC risk from beef. The team ultimately aims to reduce human STEC cases and outbreaks from beef, while preserving an economically viable and sustainable beef industry. The effort focuses on the STEC that are classified as adulterants in beef by the USDA, Food Safety and Inspection Service, viz., O26, O45, O103, O111, O121, O145, and O157:H7; in addition, it addresses enteroaggregative STEC O104:H4. These eight serogroups and serotypes of STEC (STEC-8) are addressed in five main objectives: (1) STEC-8 detection—reagents, sampling plan, assays, technology, partners; (2) STEC-8 microbiology, eco-epidemiology, exposure risk, intervention targets; (3) Interventions for STEC-8 risk reduction—value, feasibility, cost-benefit, impacts; (4) STEC-8 risk analysis and risk assessment; (5) Risk management and risk communication through beef chain STEC-8 translational education, outreach and evaluation.

S8 Big Data: Food Safety's Holy Grail or Pandora's Box?

PAJAU VANGAY: *University of Minnesota, Minneapolis, MN, USA*
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The question is simple, but not easy: is food safety ready to harness big data? The three Vs are commonly used to describe "big data"; Volume: the sheer amount of data being generated; Variety: the different data types that have to be consolidated; and Velocity: the speed at which this data is output. At the dawn of the information age, there are over 1.5 billion online searches per day and 3,000 tweets per second. The FDA, CDC, UC Davis, and others have partnered to sequence 100,000 foodborne pathogen genomes. Along with other publicly available genomics databases, these resources will provide valuable information that can be used to improved detection of foodborne illness outbreaks as well as identification of sources responsible for pathogen and spoilage organism contamination. Moreover, the food industry worldwide conducts millions of food safety tests that are never harnessed in a collaborative manner.

This symposium will discuss the applications of big data to food safety and address questions concerning the potential benefits and challenges associated with data collection, storage, analyses, and sharing. Big data has not been a symposium topic covered previously by IAFP to our knowledge. Furthermore, its potential applications and hope on how it might be used to advance food safety and save lives across all sectors in the food system warrant further discussion. This symposium will address this concern and provide ideas for companies on how to utilize big data.

The use of big data will require a cultural shift from independent advancements to collective advancements. It will require the sharing of data, thus allowing for data to be more transparent and usable. Lastly, in the global food system, the ability to make quick and sound decisions related to food safety is imperative. Big data has the promise to help food safety professionals make better informed decisions because of the knowledge that can arise from harnessing big data.

S9 Global Consumer Food Safety Practices for Raw Poultry and Shell Eggs: Areas for Improvement

SHERYL CATES: *RTI International, Research Triangle Park, NC, USA*
KADRI KOPPEL: *Kansas State University, Manhattan, KS, USA*
EDGAR CHAMBERS: *Kansas State University, Manhattan, KS, USA*
CHRISTINE BRUHN: *University of California-Davis, Davis, CA, USA*
ELLEN EVANS: *Cardiff Metropolitan University, Cardiff, United Kingdom*
JENNIFER QUINLAN: *Drexel University, Philadelphia, PA, USA*

A major source of consumers' exposure to *Salmonella* and *Campylobacter* is from poultry and shell eggs. These products are consumed frequently in the U.S. and worldwide thus the potential for exposure is great. Recent surveys and other research suggests that many consumers do not follow recommended food safety practices; however, limited research has been conducted specifically related to consumer purchase, storage, handling, and preparation of raw poultry and shell eggs. This symposium presents findings from multiple USDA Agriculture Food and Research Initiative (AFRI) grants and other research on current food safety practices for raw poultry and shell eggs in the U.S. and other countries and identifies areas for improvement. This symposium will feature five sessions, and conclude with a panel discussion.

First, findings from a nationally representative survey of U.S. adults (n = 1,500) will be presented to describe how consumers purchase, store, handle, and prepare raw poultry and shell eggs. The survey over-sampled ground poultry product users to learn how consumers prepare this product, in particular, determining doneness. Next, the findings from this U.S. survey will be compared to practices in Argentina, Columbia, Spain, Italy, Estonia, Russia, India, Thailand, and Korea. Then, the results of an observational study related to behaviors for purchasing and storing raw poultry will be presented from 97 "shop-a-long" interviews. Next, results of two observational studies will be presented to illustrate common mistakes made by U.S. and United Kingdom consumers when cooking raw poultry. Then, results of a study conducted to educate consumers on a common unsafe practice, washing raw poultry before cooking, will be presented. The session will conclude with a panel discussion on translating research findings into action and how to motivate consumers to change unsafe practices for poultry and shell eggs.

S10 Bacterial Sporeformers: A New Look at Some Old Foes and the Challenges They Pose to Today's Foods and Beverages

ALEXANDER MATHYS: *German Institute of Food Technologies DIL, Quakenbrueck, Germany*
SARAH MARKLAND: *University of Delaware, Wilmington, DE, USA*
ERIC JOHNSON: *University of Wisconsin-Madison, Madison, WI, USA*

Due to their extreme resilience, longevity, and resistance to heat and chemicals, the endospores of pathogenic and spoilage bacteria from the genera *Bacillus*, *Brevibacillus*, *Clostridium*, *Desulfotomaculum*, *Geobacillus*, *Alicyclobacillus*, *Paenibacillus*, and *Sporolactobacillus*, pose unique control challenges for the food industry. These sporeformers have been associated with illness or spoilage of a wide variety of ready-to-eat food and beverage products (distributed at ambient, chilled, or hot-vend temperatures), and their control strategies require a good understanding of contamination sources, sporulation, germination and outgrowth mechanisms, as well as conventional and novel methods of destruction. This symposium will provide an update on the unique characteristics of spores, examine recent spore-related food safety events, and discuss re-emerging issues associated with psychrotolerant sporeformers. Developments in non-traditional inactivation treatments for the control of endospores will be addressed, as well as recent work on attachment, multicellular behavior, and biofilm formation of endospore-forming bacteria.

S11 Parasites in the Food Supply Chain: Emergence or Re-emergence?

YNES ORTEGA: *University of Georgia, Griffin, GA, USA*
PALMER ORLANDI: *U.S. Food and Drug Administration-CFSAN, Silver Spring, MD, USA*
REBECCA HALL: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Foodborne parasites have not received the same level of attention as other bacterial foodborne pathogens. However, recent recalls/outbreaks due to foodborne parasites has raised the need to re-evaluate the prevalence, control, transmission and globalization of the food trade on the role of parasites in the food supply chain. This symposium will provide an overview of the types of foodborne parasites, the mode of transmission, ecology, host relationship prevalence, transmission mitigation strategies and survival in food and water. In addition, this symposium will review a case study on the recent *Cyclospora* outbreak.

S12 Integral Role of the Microbiome in Food Safety and Human Health

PATRICIA HIBBERD: *Harvard University, Boston, MA, USA*
S. STEVE YAN: *U.S. Food and Drug Administration, Rockville, MD, USA*
SUSAN KOTARSKI: *Zoetis, Kalamazoo, MI, USA*
SANGEETA KHARE: *U.S. Food and Drug Administration, Jefferson, AR, USA*
BRUNO SOBRAL: *Nestle, Lausanne, Switzerland*
JENNIFER PATRO: *U.S. Food and Drug Administration, Laurel, MD, USA*

Over the past few years, much attention has been directed to the role of the human gut microbiota as it relates to human health. In addition, there have been a number of studies to indicate that these microbial communities are affected by the foods, i.e., nutrition, toxins, and associated microbes present, we consume. This symposium is intended to address several facets on this developing area of research and interest. These include topics as (1) foods provide nutrition to humans as well as introducing whatever microbial population that is present in that commodity, whether beneficial, neutral or pathogenic; (2) the affect of antimicrobials on the microbiota and the metabolism of antimicrobials by the microbiota in an individual; (3) how the microbial communities in the human play a major role in the host-pathogen interactions; (4) present some of the current technologies used in microbiome research; (5) the analysis of the gut microbiome by food additives and probiotics; and (6) where we are in regard to the current and future state of microbiome studies.

S13 Advancing Worldwide Laboratory Quality Culture and Food Safety through Effective Proficiency Testing and Data Analysis

CARL SCIACCHITANO: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
MANFRED STOYKE: *Federal Office of Consumer Protection and Food Safety, Berlin, Germany*
MARIA TERESA DESTRO: *University of Sao Pãulo, Sao Pãulo, Brazil*
TAN LING: *China National Accreditation Service, Beijing, China*
STEFFEN UHLIG: *Quo Data, Dresden, Germany*

Laboratory Quality Culture (LQC) is a culture throughout the analytical laboratory that continually views quality as a primary goal and views tools of analytical quality assurance such as proficiency testing (PT) not only to identify dysfunctions and incompetence but also as tools to learn and to improve processes and analytical methods. Proficiency testing is the determination of testing performance by means of inter-laboratory comparisons. The Food Safety & Modernization Act (FSMA) contains several directives that align with LQC and PT. Section 305 of the Act mandates development of a "comprehensive plan to expand the technical, scientific, and regulatory food safety capacity of foreign governments, and their respective food industries, from which foods are exported to the United States." LQC and PT will be a critical and significant part of compliance to FSMA. Proficiency testing with affect several sections of the Act such as (1) support laboratory accreditation, (2) building domestic and international laboratory capacity, (3) surveillance, (4) training, and (5) development of laboratory standards. In addition, the role of PT in each country varies, such as (1) controlling market access, (2) accreditation, (3) training, (4) internal AQC, (5) method performance, (6) reference materials, (7) calculation of measurement uncertainty and, (8) dissemination of lab quality culture. Through an effective PT and data analysis, comparability and equivalence of laboratory data can be obtained and used to assess the efficiency of the tested labs. Therefore, one of the objectives of this symposium is to bring speakers from U.S., EU, South America, China that can address international perspectives and present case studies that affect global food safety. This symposium will function as a network of national and international PT organizations that drive laboratory quality culture to ensure a safe, worldwide food supply.

S14 Emerging, Re-emerging and Opportunistic Foodborne Pathogens: Bugs You Don't Know May Bug You!

MARTA MARTINS: *University College Dublin, Dublin, Ireland*
PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
YNES ORTEGA: *University of Georgia, Griffin, GA, USA*
DAMIEN JOLY: *Metabiota, Nanamio, BC, Canada*
PINA FRATAMICO: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
KEITH LAMPEL: *U.S. Food and Drug Administration, College Park, MD, USA*

To many food safety professionals, the significance of emerging, re-emerging and soon to be emerging microbial pathogens has become an obvious critical issue as we address the consumer's focus on public health. In several surveillance studies, these pathogens have been collectively grouped in the category of unspecified etiology. This may be due to inability to identify these microbes for a variety of reasons such as the physiological state of the pathogen or a lack of a robust isolation method from foods. However, in some cases, we may never have anticipated a particular microbe to be associated with food or the creation of a mosaic hybrid pathogen such as *Escherichia coli* O104:H4.

The Food and Agriculture Organization (FAO) of the UN has indicated that 60 percent of all emerging infectious diseases have zoonotic sources. Therefore, the global food supply may act as not only a source of disease causing organisms but also has an in vivo medium to generate novel pathogens. Consequently, the broader picture of risk associated with the consumption of contaminated foods can be delineated and managed only if the biological hazards (specifically food borne pathogens) linked to food are well known and studied. If one considers the scenario that the biological hazard associated with food was never anticipated or even unknown, the impact of this lack of data can have significant consequences in many facets of food safety as it pertains to regulations, policy, and the food industry. There is an eminent need to acquire data related to emerging and re-emerging pathogens to translate the biological hazards from an unknown origin to a known entity. This symposium will address the critical issues about emerging, re-emerging and soon to be emerging agents in a similar way as other well known foodborne pathogens. Speakers have been asked to address the long-term research need to improve our ability to respond quickly to new microbial threats and help us become more proactive at anticipating and preventing emergence.

S15 Restaurant Food Safety Risks: Ill Workers, Leafy Greens Preparation Practices, Microwave Practices, Food Cooling Practices, and (Lack of) Kitchen Manager Certification

CAROL SELMAN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DAWN NORTON: *California EIP, Oakland, CA, USA*
KRISTIN DELEA: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
NICOLE KOKTAVY: *Minnesota Department of Health, St. Paul, MN, USA*
DONALD SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
LAURA BROWN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Half of all foodborne illness outbreaks are associated with restaurants. To better understand the environmental causes of restaurant-related foodborne illness outbreaks, and subsequently reduce or mitigate them, the Centers for Disease Control and Prevention's (CDC) Environmental Health Specialists Network (EHS-Net) conducts studies on food preparation practices and other factors that could contribute to foodborne illness outbreaks. Speakers at this symposium will present results from five such studies and discuss them within the framework of foodborne illness outbreaks. Specific findings to be discussed include the following.

- Many restaurants do not have policies that specifically address when and for how long ill workers should be prevented from working.
- Many restaurants engage in leafy greens preparation practices discouraged by the Food and Drug Administration (FDA), such as soaking leafy greens during washing and using bare hand contact while preparing leafy greens.
- Some restaurants do not take the precautions to ensure that foods cooked in microwaves reach safe temperatures.
- FDA recommended food cooling practices positively impact food cooling rates.
- Food safety certification positively influences restaurant manager and worker food safety knowledge.

S16 What is Slowing Down Rapid Methods? Sample Prep!

LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
PALMER ORLANDI: *U.S. Food and Drug Administration, Rockville, MD, USA*
KAREN JINNEMAN: *U.S. Food and Drug Administration, Bothell, WA, USA*
MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium*
ANJA BUBECK-BARRETT: *Roka Bioscience, Inc., San Diego, CA, USA*
SCOTT HOOD: *General Mills, Inc., Golden Valley, MN, USA*

Sample preparation is a critical component of any successful protocol to detect, identify and isolate microbial pathogens in food commodities; however, more attention and resources are funneled to increasing the speed and throughput of high technology diagnostic instruments, while sample preparation remains an underdeveloped process. In recent years some sectors of the food safety community have recognized the importance of sample preparation methods, and this symposium will provide an update on this critical component of foodborne pathogen analysis. Speakers will address the major hurdles for achieving an effective and efficient means to process food samples. A brief overview of protocols currently used will present a picture of food safety at the beginning of the process. Research needs for sample prep method improvements will be highlighted. Lastly, there are those foodborne pathogens, e.g., *Cyclospora, Cryptosporidium* and norovirus, that, at this time, are not culturable and for which sample preparation is both the crux of the method and the major impediment for detection. Are we closer to resolving this critical dilemma?

S17 Do You Know What You're Getting?: Managing Chemical Hazards Associated with Imported Foods and Ingredients

SAMUEL GODEFROY: *Health Canada, Ottawa, QC, Canada*
PAUL HANLON: *Abbott Nutrition, Columbus, OH, USA*
JOSEPH SCIMECA: *Cargill, Inc., Wayzata, MN, USA*

Chemical contamination in foods can occur at any stage of the food chain. It can happen at the primary production stage due to environmental pollution, the presence of naturally occurring toxins, or the use of various chemicals during production. Harmful chemicals can also be introduced as a result of food processing or migration from packaging materials.

Managing the risk associated with chemical hazards can be complex because different foods or ingredients may be impacted by different types of chemical contaminants. The issue can be further complicated when dealing with import as the regions where the foods/ingredients are produced may have high levels of environmental contamination, may lack a basic understanding of food safety practices, or, in some cases, have different food safety standards.

In response to these challenges, government agencies and food importers have been vigilant in developing monitoring and supplier control programs to minimize the negative public health impact associated with chemical hazards in foods. This symposium will highlight current and emerging chemical food safety issues and showcase innovative tools and criteria that food companies are using to assess the potential risks that may be associated with imported foods/ingredients and to design risk-based supplier control programs. The symposium will also highlight how some companies have taken a more proactive approach to work in partnership with foreign suppliers to implement modern food safety management systems and ensure international standards are met.

S18 The Problem of Mites in Foods

BARRY O'CONNOR: *University of Michigan, Ann Arbor, MI, USA*
MARIO SANCHEZ-BORGES: *University of Venezuela, Caracas, Venezuela*
RACHEL PEARSON: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Stored product mites have long been a problem because food quality suffers. Infestations can cause an increase in mold proliferation as well as changes in a product's organoleptic characteristics. In addition, consumption of mite contaminated products can lead to negative health effects including allergic reactions. Stored product mite proteins can be chemical hazards because they can cause IgE-mediated reactions following consumption. The potential hazard from allergens produced by food-contaminating pests is a serious emerging health issue. Moderate to severe allergic reactions following the consumption of mite contaminated foods have been reported worldwide including, the United States, Spain, Venezuela, Brazil, Singapore, Japan, and Taiwan. There are currently 20 recognized storage mite species that produce allergenic proteins. These mites have infested numerous commodities including grains, flours, dried fruit, dried fish and cheese. Recent case studies (139) have highlighted the sometimes fatal effects of consuming mite contaminated food. The economic impact of mites in foods can also be significant. When mites are left uncontrolled their damage can lead to huge economic losses; cheese producers have reported losses of 10–25 percent. Recent data on mites collected from infested cheese have shown population numbers well above the level of what has been reported to cause adverse health effects. In response to both health and economic concerns, regulations have been established to protect some particularly hard hit commodities. The topics covered in this symposium include: the food products mites are more likely to contaminate, the favorable conditions that allow mites to proliferate and the ways in which they can contaminate food, case studies of allergic reactions being reported by the medical community, and the basis for current regulations. All of these topics add to the discussion about mites in foods and whether or not they are the "tiny little problem" that some believe.

S19 Modernizing Government Food Control Systems: New Tools and Core Elements

GREG READ: *Australian Government Department of Agriculture, Canberra, Australia*
MARISA CAIPO: *Food and Agriculture Organization of the United Nations, Rome, Italy*
ANA MARISA CORDERO: *Interamerican Institute for the Cooperation of Agriculture, San Jose, Costa Rica*

Can we create a model for national food regulatory systems? As the world grows smaller and food is shipped farther, consumers and importing companies are relying on control systems in use in many other countries. But who can we trust?
The Food and Agriculture Organization of the United Nations and Codex Alimentarius have developed new tools for national governments around the world to evaluate and improve their own systems. There are also several tools in use in different regions and sectors. This session will examine these tools.

S20 Collaboration on Solutions for Food Safety Advancement in China

XIUMEI LIU: *Chinese National Center for Food Safety Risk Assessment, Beijing, China*
CARL SCIACCHITANO: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
CINDY JIANG: *McDonald's Corporation, Oak Brook, IL, USA*
JULIA BRADSHER: *Global Food Protection Institute, Battle Creek, MI, USA*

China remains an important player in the food chain as the globalization of food supply continues to grow rapidly. Through internal and external efforts, mostly, in the form of collaboration, China's food safety has made significant improvement in recent years. US government agencies and food safety professionals have been working closely with Chinese government, industry, and academia in building regulatory infrastructure, enhancing food safety risk communication, and improving research, education, and technology transfer. These collaborations have positively impacted China's food safety. This symposium will bring together leading experts from government agencies, industries, and academia to share their experiences and perspectives. The objectives of this symposium are to enhance the understanding of China food safety status and programs, and to provide insights on solutions for food safety advancement in China for those who are interested in working and collaborating in China.

S21 Revisiting Enterohemorrhagic *Escherichia coli* and the *stx*2 Toxin

JOHN BESSER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ALISON O'BRIEN: *University of the Health Sciences, Bethesda, MD, USA*
ALISON WEISS: *University of Cincinnati, Cincinnati, OH, USA*

Foodborne illness outbreaks associated with enterohemorrhagic *E. coli* (EHEC) have not abated, and additional serogroups (in addition to the "big six" non-O157 STEC) are being associated with foodborne illness. A 2011 outbreak of EHEC in Germany, associated with fresh sprouts, sickened thousands and killed over 50 people as a result of contamination with serogroup O104 – a strain characterized as an enteroaggregative-EHEC that had acquired the gene that encodes for Shiga toxin-2 (Stx2) and appeared to be more virulent than O157:H7. EHEC-associated illnesses inducing hemolytic uremic syndrome (HUS) typically result from the very potent Stx2. Foodborne illnesses resulting only from Stx1, and not Stx2, are typically less severe and shorter in duration. Many food safety professionals are unaware of this distinction. A plan to develop a vaccine to protect individuals at high-risk (including laboratory researchers) against Stx2 has been proposed. Food safety professionals are occasionally asked for their non-medical opinion on the use of antibiotics to treat EHEC. In most EHEC-associated illnesses, antibiotics are contraindicated, yet they are still sometimes administered. This symposium will discuss these issues and help food safety professionals better understand and respond to questions related to Stx2-producing strains of *E. coli.*

S22 Impact of Produce Safety Rules for Sprout Growers

MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
ROBIN KALINOWSKI: *Illinois Institute of Technology, Bedford Park, IL, USA*
KRISTIN COLONY: *The Vista Institute, Eden Prairie, MN, USA*

To improve sprout safety, the U.S. Food and Drug Administration (FDA) is proposing a number of requirements for commercial sprout growers in the proposed Produce Rule. The impact of these proposed requirements on sprout growers, large or small, remains to be determined. The FDA, cooperating with the Illinois Institute of Technology's Institute for Food Safety and Health (IIT IFSH), has created the Sprouts Safety Alliance (SSA) to help sprout producers in identifying and implementing best practices in the safe production of sprouts. The Alliance, composed of members representing sprout producers, federal and local government officials and academia, is developing a core curriculum and training programs for stakeholders in the sprout production community to promote adoption of current best practices now and to assist sprout growers in complying with the FDA's Produce Rule, when final. In this symposium, a review of the FDA's proposed produce safety rule will be given as it relates to the sprout producers. Educational information on cleaning, sanitation and irrigation water testing developed by the Alliance will be discussed as it relates to sprout growers. Commercial sprout growers will give their perspective as how the Produce Rule will affect them.

S23 First-hand Accounts of Foodborne Illness: Physicians, Parents and Patients Speak Out

BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
NANCY DONLEY: *STOP Foodborne Illness, Chicago, IL, USA*
DANA DZIADUL: *Wake Forest, NC, USA*
CHRISTIANE HADI: *Immunizations and Refugee Health, Indianapolis, IN, USA*
PATRICIA BUCK: *Center for Foodborne Illness Research and Prevention, Raleigh, NC, USA*

Food safety professionals have a vested interest in improving food safety and preventing foodborne outbreaks. Nevertheless, patients who suffer from foodborne illnesses, as well as their families, often appear only as numbers or statistics to those professionals who most greatly impact food safety on a day-to-day basis (*e.g.*, research scientists, government officials, epidemiologists, and food industry management personnel). The reality and personal tragedy accompanying a severe foodborne illness is intensely physiological, emotional, and psychological, affecting the patient, their family, friends and co-workers. Oftentimes, humans do not become aggressively motivated to remediate a disease until they see the dark and painful "human side" of an otherwise list of data and statistics. Only after a person sees through the eyes of the patient, the physician, or the grieving parent, does the illness take on new meaning – increasing their resolve to find solutions and cures to the excruciatingly painful scourge of human suffering.

This symposium will provide (for many attendees) new insight and perspective into the pain that is experienced in the wake of a foodborne outbreak. Attendees will hear first-hand accounts of the physiological, emotional, and psychological outcomes of foodborne illnesses. The intent of this symposium is to both inform and inspire food safety professionals as to the extremely valuable nature of their work – with the goal of motivating IAFP attendees to excel in their fields; hopefully preventing future foodborne illnesses.

S24 Cleaning and Sanitation of Low-water Activity Processing Environments

STEVE BLACKOWIAK: *Bühler Aeroglide, Raleigh, NC, USA*
NICOLE DELANEY: *Ecolab Inc., Eagan, MN, USA*
KELLY STEVENS: *General Mills, Inc., Golden Valley, MN, USA*

In the last five years, there have been ten foodborne outbreaks associated with low-moisture foods including nuts, spices, pet food, cookie dough, and extruded snack foods. The causative agents of these outbreaks include *Salmonella* and enterohemorrhagic *Escherichia coli*, and were linked to over 1,600 cases of foodborne illness and nine deaths. As pathogens are able to persist in low water activity products and processing environments it is imperative to employ mitigation strategies to minimize pathogen occurrence. Low water activity (a_w) processing environments present different food safety challenges than those found in higher a_w processing environments. In the case of low a_w processing, introduction of water can lead to an increased problem, allowing microorganisms to proliferate. Food processing equipment and nonfood-contact surfaces must be cleaned prior to sanitation. This creates a dilemma for manufacturers of low a_w products, as cleaning techniques typically utilized in the food industry involve the use of hot water and detergent. Dry cleaning techniques are dependent on the level of clean desired and the rationale behind performing the cleaning procedure. Dry sanitation techniques can then be used to provide targeted microbial inactivation and specify 'lot' separation. Special care must be taken to ensure the hygienic design of new processing equipment. In the case of older processing equipment, retrofitting may be employed to aid in cleanability. To ensure a pathogen-free processing environment, validation and verification procedures must be developed and implemented.

S25 Less Known or Under-utilized Approaches to Dry Cleaning and Sanitation

JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
JOEL WILLIAMS: *Precision I Splash Corporation, Peshtigo, WI, USA*
GARY GOESSEL: *Kellogg's, Battle Creek, MI, USA*

Recognition of microbiological risks associated with low water activity foods is increasing as evidenced by recent events including a large multistate recalls of hydrolyzed vegetable protein, peanut butter, spices, and dry milk, nut meats and others. Many of these dry products are often ingredients in other products and can consequently drive further recalls of those products. High water activity food producers were and are still driven by economic constraints to seek equipment designed in a hygienic manner that can accommodate moisture. This is because such high water activity products spoil quickly with attendant loss of desirability and market share. These incentives have historically not existed with low water activity foods because microbiological spoilage does not occur. Consequently, equipment used to manufacture such foods is often not designed in a manner to accommodate water used in wet cleaning and sanitization. In fact, wet cleaning has been shown to increase the risk of pathogen contamination of low water activity foods. However, low water activity food processors are under increased pressure to perform more frequent wet cleaning and sanitization due to considerations related to allergen changeovers and regulatory pressures. Hence the need exits for technologies that can be applied to effectively clean and sanitize low water activity food processing environments and equipment.

S26 Advances in Risk Assessment and Modeling Tools and Their Practical Application in Food Safety

ALEJANDRO AMEZQUITA: *Unilever, Sharnbrook, United Kingdom*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece*
YUHUAN CHEN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
MATTHIAS FILTER: *Federal Institute for Risk Assessment, Berlin, Germany*
MARISA CAIPO: *Food and Agriculture Organization of the United Nations, Rome, Italy*
MAARTEN NAUTA: *DTU Food, Copenhagen, Denmark*

In recent years, new computing tools for food safety, quality assessment and for decision-making have been developed and made globally available by industry, academia, international organizations and governments. These new tools, usually free downloadable or web-based applications, cover a broad range of domains including the development and validation of sampling plans, the prediction of the behavior of microbes in food (predictive microbiology), and the development of probabilistic risk assessment and risk ranking models. This symposium draws upon speakers from international organizations, government, industry and academia to discuss common and specific needs regarding these tools. Practical applications of the currently available tools will be presented. Discussions on recent advances and existing gaps in data and modeling capacity will facilitate an open dialogue on the challenges and opportunities for development of future tools. It will also help determine what can be done to further improve these tools to meet the needs and expectations of industry and decision makers.

S27 Stressed Out! A Microbial View of Life in the Food Production Environment

BYRON BREHM-STECHER: *Iowa State University, Ames, IA, USA*
MANAN SHARMA: *U.S. Department of Agriculture-ARS, Beltsville, MD, USA*
SEAMUS FANNING: *University College Dublin, Dublin, Ireland*
KEVIN ALLEN: *University of British Columbia, Vancouver, BC, Canada*
MONICA PONDER: *Virginia Tech, Blacksburg, VA, USA*
SIDDHARTHA THAKUR: *North Carolina State University, Raleigh, NC, USA*

The food production environment imposes a variety of microbial stresses on bacteria, both physical and chemical. Suboptimal temperature, pH, Aw, as well as the presence of chemical preservatives, antimicrobials, and sanitizers, are factors that can cause sublethal physiological stress, resulting in altered physiological responses by microbial cells. Despite the stresses, microorganisms can survive, contaminate food products and potentially cause illness to humans. How do they survive these suboptimal conditions? There has been a long history of study of injured or stressed microorganisms, with the aim of developing appropriate methods to ensure their detection. Recently, advances in molecular biology methods have improved our ability to identify the mechanisms by which microorganisms can survive stresses encountered in the food production environment. Can bacteria adapt to these stresses, and persist in food production environments after exposure? This symposium will examine responses of foodborne pathogens to various stresses encountered in the food production environment and will reveal how an understanding of the mechanisms of stress survival can lead to more effective ways to control pathogens in foods.

S28 The Acid Test: Metabiotic Effects and the Safety of Acid and Acidified Foods

FRED BREIDT: *U.S. Department of Agriculture-ARS, Raleigh, NC, USA*
ANTONIO BEVILACQUA: *Foggia University, Foggia, Italy*
DANIEL GEFFIN: *U.S. Food and Drug Administration, College Park, MD, USA*

The focus of this short symposium is on metabiotic effects and food safety significance of spoilage microorganisms that can increase the pH of acid and acidified food products, resulting in more favorable growth environments for pathogenic microorganisms. Acid-resistant pathogens can survive and cause disease in acidified foods, such as apple cider, orange juice, and some pickled vegetable products that have pH values below 4.0. Recent FDA guidance has raised concerns about spoilage molds (such as *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp.) and bacteria (such as *Bacillus licheniformis*) that can increase the pH of acidified foods and beverages, potentially resulting in a metabiotic effect that allows spore germination, growth, and toxin production by *Clostridium botulinum*. Several studies in home-canned tomatoes and tomato juice have supported this hypothesis. Certain metabiotic associations between foodborne molds and pathogenic bacteria have been observed in fresh produce, as well as in mold-ripened cheeses. Molds have been reported to enhance the growth and survival of pathogens by changing their growth environments. These concerns are addressed by both FDA guidelines and the Food Safety Modernization Act. Given the proliferation of novel fermented and acidified products and imported foods, there is a pressing need for better understanding of processing conditions that will assure safety and support regulatory compliance.

S29 Buyer Beware: Intentional Adulteration Can be a Major Food Safety Concern

COLIN BARTHEL: *U.S. Food and Drug Administration, College Park, MD, USA*
JOHN HOFFMAN: *University of Minnesota-NCFPD, St. Paul, MN, USA*
GALE PRINCE: *Sage Food Safety Consultants, Cincinnati, OH, USA*

Intentional adulteration can come from different forms and for different reasons. It may be done for economic gain or for economic damage to a food company or for criminal damage to society.
Historically the most common form of intentional adulteration has been for economic reasons through substitution, dilution, enhancement of appearance or misbranding. But it has also involved diversion, transshipping and theft to feed a gray market. Intentional product adulteration has a dark side with attempts to cause economic adulteration to individuals or companies. It has also resulted in deaths of humans and animals. This is a global issue. Examples of economic adulteration includes juices, pet food, baby formula, seafood, oils, honey, spices, extracts, grain products, alcoholic beverages to name a few.

Intentional adulteration can be silent long term actions to high profile news events with public threats of contamination or false reports that can damage public confidence in product safety. Acts of intentional adulteration can take place at any point in the food distribution chain and can be from an internal source or from an external source. While these cases have been limited there is a history of individual prosecutions and fines leveled against companies but economic burden is far greater for companies and the public.
United States Congress included a requirement in the 2011 Food Safety Modernization Act (FSMA) requiring the Food and Drug Administration to issue regulations and guidance on intentional adulteration. Preventive controls are key elements in protecting a firm from being a victim of intentional contamination and, more importantly, the consumer.

This symposium will highlight the impact of food adulteration what industry and regulators are doing to minimize the risks of adulteration in the food chain, especially FSMA requirements for innovative methods to detect adulteration and for targeting crucial resources toward the riskiest food products.

S30 Transitioning Research and Integrating Tools to Build a Comprehensive Food Protection System

AMY KIRCHER: *University of Minnesota, St. Paul, MN, USA*
COLIN BARTHEL: *U.S. Food and Drug Administration, College Park, MD, USA*
JOSEPH SCIMECA: *Cargill, Inc., Minneapolis, MN, USA*

The food and agriculture sector is a globally distributed and highly integrated system of systems. Threats to the food and agriculture sector come in many forms from natural disasters to intentional contamination for economic, criminal or terrorist reasons. The complexity of this system makes rapid identification of an assault to the system a difficult challenge.

Traditionally the identification of an adverse food contamination event comes from clinical and public health data collected and reported only after individuals have consumed the product and become sick. Similarly, the contaminated food item and its distribution are often identified after the event itself. Consequently, by the time a food item has been implicated as the source of the contamination, a significant portion of the potential target population may have already consumed the product.

The recent threats from weapons of mass destruction, continual identification of economically motivated adulteration, and the Food Safety Modernization Act have served as motivation to create and implement innovative technologies for stakeholders in the food system (public and private) to identify threats, assess risk, and mitigate consequences of an adverse event in the food system. The government, private sector, and academia have transitioned research into tools and capabilities to mitigate the consequences of food system disruptions. This session will offer not only an overview of the tools but also how they integrate to build one comprehensive food protection system. Symposium speakers will come from academia, a government organization with food regulation responsibilities, and the private sector.

S31 Microgreens and Sprouts under Microscope: Similarities and Differences in Botanic Structure, Agricultural Practices, and Food Safety Risks

DAVID SASUGA: *Fresh Origins, San Marcos, CA, USA*
XIANGWU NOU: *U.S. Department of Agriculture-ARS, Beltsville, MD, USA*
MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
YAGUANG LUO: *U.S. Department of Agriculture-ARS, Beltsville, MD, USA*

Recent foodborne illness outbreaks associated with consumption of sprouts have heightened consumers' food safety concerns regarding this commodity. Perceived as a potential alternative, microgreens have become increasingly popular. Microgreens are usually a few days older than seed sprouts, can be grown hydroponically, or in soils, and are harvested without the roots. The proposed FSMA produce safety rule requires special seed treatments and other requirements for growing sprouts due to their unique food safety risks. However, whether this same rule should apply to microgreens is unclear. This proposed symposium will bring together leading researchers, growers, and regulators in this field to share their knowledge, experience, and perspectives. The outcome of this symposium will be a clearer understanding of the differences/similarities of sprouts vs. microgreens in terms of their botanical structures, agricultural practices, and food safety risks, thus contributing to the development of future food safety regulations

that are risk-based, and enforceable. Furthermore, information gathered can also be used by microgreen and sprout growers to optimize their operations in order to further improve the safety of these otherwise nutritious food products.

S32 Microbiological Hazards in Veal Slaughter: Identification of Contributing Factors

RANDALL PHEBUS: *Kansas State University, Manhattan, KS, USA*
MOHAMMAD KOOHMARAIE: *IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*
BOB RUSSELL: *Marcho Farms, Harleysville, PA, USA*

The Food Safety and Inspection Service (FSIS) recently expanded its beef trimmings testing program to include the six adulterant non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Recent findings show an increased percent positive rate for STEC in trimmings produced from veal as compared to trimmings produced from other cattle slaughter classes. This session will focus on scientific research achievements and identification of contributing factors to adulterant STEC in veal, including pre-harvest controls, weaning practices, best practices in slaughter establishments, and antimicrobial interventions. Discussion of these findings is intended to stimulate further academic, industry, and regulatory agency collaborations to explore current research needs. Additionally, the meeting is intended to inform regulators and the veal industry on risk management strategies based on the identified risk factors. USDA/FSIS staff, STEC Coordinated Agricultural Project (CAP) grant and other researchers, and industry representatives will focus on risk factors based on research findings and discuss risk mitigation strategies based on the findings. The session will be research-based and will not focus on policy or economics.

S33 Food Industry on the Media Hotspot: How Have They Recovered from Crisis Situation?

JOHN RUBY: *JBS, Green Bay, WI, USA*
ROGER COOK: *New Zealand Food Safety Authority, Wellington, New Zealand*
SAMUEL GODEFROY: *Health Canada, Ottawa, ON, Canada*

Food safety best practices have been long adopted by the food business operators, following HACCP principles and continued quality management. Most of the companies have also applied food safety standard such as IFS (International Food standard), BRC (British retail Consortium), and ISO 22000. Based on these standards, the food standard setters have included chapters about crisis management and product recalls to help the food industry demonstrate their management of crisis situations. Putting theory aside, many of the Food Business Operators have had to face crisis management, involving their products in outbreak situations. This symposium will present real case studies faced by the food industry, and will combine different situations and different food industry. How did they manage the crisis situation? How did they recover? What have they learned? How have they improved their best practices?

S34 Developing a Protocol for Meeting FSMA Validation Requirements in Heat Processed Low-moisture Foods

NATHAN ANDERSON: *U.S. Food and Drug Administration/IFSH, Bedford Park, IL, USA*
DAVID ANDERSON: *Del Monte Foods, Pittsburg, PA, USA*
CARRIE FERSTL: *The National Food Laboratory, Inc., Livermore, CA, USA*
BRADLEY MARKS: *Michigan State University, East Lansing, MI, USA*

Compliance with FSMA's validation requirements, especially in low-moisture foods manufacture, will necessitate significant collaboration among all stakeholders – food processors, equipment manufacturers, ingredient suppliers, laboratories, regulators, associations, and academia – in order to provide the kind of data regulators will be looking for in process validations. Food processors and equipment manufacturers especially play a key role in this validation process in the low-moisture foods arena.

Because *Salmonella* may survive low-moisture conditions and may grow if a processing facility is unable to effectively manage the introduction of water, low-moisture products are an emerging food safety challenge. Implicated low-moisture foods can include chocolate, cocoa, confectionary products, dried milk, tree nuts, peanuts, peanut butter, flours, cereals, spices, pet treats, and other foods.

To address these food safety concerns, the Product Safety Solutions Group of the Alliance for Innovation & Operational Excellence (AIOE) recently published a guidelines document – Validating the Reduction of *Salmonella* and Other Pathogens in Heat Processed Low-Moisture Foods – for the multiple stakeholders involved in the safe delivery of these products to consumers. Additionally, AIOE's Engineering Solutions Group will soon publish a work product, a collaborative effort by food processors and equipment manufacturers, on the "must have" guidelines for minimum food safe sanitary design of equipment in low-moisture food manufacture.

This session introduces key topics from these guidelines and will focus on validation of processes and standardized methods to report findings, implementing process controls, conducting verification activities, and documenting control measures in food safety plants. By expanding awareness of this "state-of-the-art" guideline document beyond the immediate food processor and equipment manufacturer community, the session provides high value for IAFP attendees in this critical food safety area and demonstrates the effectiveness of partnership among stakeholders to develop effective options for the food industry.

S35 Understanding and Mitigating *Salmonella* Risk in Low Water Activity Foods Using Quantitative Microbial Risk Assessment

SEAMUS FANNING: *University College Dublin, Dublin, Ireland*
ELISABETTA LAMBERTINI: *University of Maryland, College Park, MD, USA*
MICHELE EVANS: *Diamond Pet Foods, Topeka, KS, USA*
JEAN-LOUIS CORDIER: *Nestec S.A., Vevey, Switzerland*

Goal: To communicate the latest research and industry practices to understand and control the risk of bacterial pathogen contamination in low water activity foods.

Abstract: Over the last several decades, a number of salmonellosis outbreaks worldwide have been associated with the consumption of ready-to-eat low water activity products including powdered infant formula, chocolate, nuts, spices, and peanut butter. Furthermore, recent outbreaks of salmonellosis associated with dry pet foods have considerably emphasized the importance of these foods as vehicles for zoonotic pathogens. Recent advances in microbial risk assessment provide the analytical framework to evaluate low water activity food systems in a systematic and quantitative way, and to inform the development of risk-based policies and strategies. The overall goal of this symposium is to provide the latest information, which includes

experimental data collection, microbial risk modeling, industry practices, on *Salmonella* control efforts in different low water activity foods including dry pet foods. Multiple industries, disciplines, and sectors will come together to discuss the latest research and industry actions to control bacterial pathogen contaminations in low water activity foods for humans and animals, from ingredients to consumption. This will range from bacterial cellular biology in low water activity environments (survival and resistance mechanisms), microbiological surveys, industry state-of-the-art practices to control pathogens, and risk modeling as an overarching tool to synthesize multidisciplinary knowledge and translate it into quantitative and actionable estimates.

S36 Molecular and Genomic Methods for the Rapid Identification of Microbes in Foods: Impact on Public Health and Food Safety

KAREN JINNEMAN: *U.S. Food and Drug Administration, Bothell, WA, USA*
SEAMUS FANNING: *University College Dublin, Dublin, Ireland*
SCOTT JACKSON: *NIST, Gaithersburg, MD, USA*
ANDREA OTTESEN: *U.S. Food and Drug Administration, College Park, MD, USA*
LARRY GOLD: *SomaLogic, Inc., Boulder, CO, USA*
NICK SICILIANO: *Invisible Sentinel, Philadelphia, PA, USA*

The holy grail of food safety is the ability to rapidly detect pathogens on and in food commodities; ideally using non-invasive technologies such as biosensors. While no such technology currently exists, researchers from government, academia and private industry laboratories are aggressively exploiting emerging molecular-genomic technologies to develop methods that allow the detection and identification of microbial pathogens on foods. This symposium will focus on six independent molecular-genomic technologies that are currently being used and/or developed for this critical aspect of food safety.

S37 21st Century Chemical Food Safety Assessment Challenges

SUZANNE FITZPATRICK: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
NORBERT KAMINSKI: *Michigan State University, Lansing, MI, USA*
AGNES FORGACS: *U.S. Environmental Protection Agency, Raleigh-Durham, NC, USA*

This symposium will address the important new initiatives that the FDA chemical risk assessment program has undertaken which will impact how FDA addresses new and recurring food safety issues. Foremost among these is the modernization of FDA's entire Chemical Food Safety Program to meet 21st century demands for both scientific expertise and transparency. The re-emergence of chemical safety as a critical food safety issue has resulted in the FDA Office of Foods conducting an in depth analysis of how the program can be modernized to meet these new challenges. Restructuring of this program across the entire FDA Foods program at FDA will be presented. Integral to this modernization is the development of a systematic approach to how FDA assesses its regulatory gaps and directs its food safety research program. Many potential changes to the chemical risk assessment paradigm will also be addressed. Using a net-effects approach for chemical contaminants, whether non-monotonic dose response issues will change how food additive safety is assessed, and the importance of evaluation of different susceptible life stages for all products regulated by FDA all have the potential to influence how FDA will conduct risk assessments in the future. Additionally efforts nationally and internationally to modernize the science of toxicology may impact on FDA's programs. The impact of Tox Cast/Tox 21 on assessing toxicity, especially GRAS and dietary supplements, is an important question that will be discussed. Additionally uptakes will be presented of several chemical compounds of concern, including inorganic arsenic, methyl mercury, and perchlorate. Taken together, the issues addressed in this symposium will inform the audience of how the FDA and the Toxicology Community are meeting the chemical risk assessment challenges of the 21st century. It will also illuminate how these challenges will impact food safety assessments.

S38 Food Traceability: Important for Food Safety and Indispensable for Food Defense

JASON BASHURA: *U.S. Food and Drug Administration, College Park, MD, USA*
TEJAS BHATT: *Institute of Food Technologists, Washington, D.C., USA*
JENNIFER MCENTIRE: *The Acheson Group, Frederick, MD, USA*

Due to the globalization of the food supply, there exists an increased risk for acts of intentional contamination for socio-economic or political gains, such as food fraud and food terrorism. In recent years there has been an upward trend towards large scale food safety incidents, resulting in significant worldwide public health and financial impacts, such as the "Horsegate" scandal or the Peanut Corporation of America outbreak and recall. The common thread that ties food safety and food defense incident planning, preparedness, response and recovery, is the ability to effectively track and trace products throughout the value chain. The fundamental question this session will explore is that if product traceability during food safety incidents (when contamination agents and suspect foods are known) is currently challenging, then what are the implications of product tracing during a food defense incident (when contamination agents or suspects foods are unknown)? Scientific, regulatory and industry speakers will present perspectives on defining the gaps and challenges associated with global food traceability, the approaches best practices to solving these problems, and the importance of an improved traceability framework to food defense.

S39 Confronting Food Allergen Trends

STEVEN GENDEL: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
SCOTT SEYS: *U.S. Department of Agriculture-FSIS, Minneapolis, MN, USA*
SCOTT HOOD: *General Mills, Inc., Minneapolis, MN, USA*
G. CRAIG LLEWELLYN: *The Coca-Cola Company, Atlanta, GA, USA*

Undeclared allergens and ingredients of public health concern in regulated food products continue to be a concern. Since 2008, the U.S Department of Agriculture, Food Safety and Inspection Service (FSIS) has recognized a notable increase in the number of recalls due to undeclared allergens with 29 in calendar year 2012. The Food and Drug Administration (FDA) has similarly seen an increasing trend over time with 189 recalls in fiscal year 2012. The goal of this symposium is to provide data, trends, and information from FDA, FSIS, and two food companies that will converge on possible mechanisms to prevent ongoing concerns.

The presentations in this symposium will provide valuable information in four principal topic areas: trends in FDA recalls due to undeclared food allergens which will help identify areas for public health improvement, results from FSIS verification activities used to gain a firsthand look at issues of concern within regulated establishments, innovation and challenges on the subject of managing allergens within food plants, and lessons learned from addressing problems, such as recalls.

S40 Striving for a True, Food Safety Culture: Going Past Training to Living

MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*
ANN MARIE MCNAMARA: *Jack in the Box, San Diego, CA, USA*
LAURA NELSON: *Alchemy Systems, Austin, TX, USA*

Food safety is not just encompassed in a HACCP plan or regulatory statues. Food safety in today's food industry is a cultural adaption that is influenced by every action and decision made in the development, production, handling, and service in the food chain. Recently, this has been demonstrated in an increased focus on making culture changes, which are illustrated in comprehensive management commitment sections in industry accepted standards. But when reality hits, leaders are often forced to solve complex challenges facing a people-driven industry like,

- How often has a person tried to make the right food safety decision, only to make a riskier choice?
- How often has a small lapse in concentration or incorrect decision lead to unsafe choices or even a food safety crisis?
- How have food companies turned the dial to make a perceptive change in their corporate culture after a crisis?
- Other than traditional training, what tangible things can be done to drive real change and continuous progress?

This session will hear from food safety experts who must answer those types of questions every day. They have developed strategies to capture employees' attention and engage them to be cognizant and legitimately concerned about making informed, smart food safety decisions in their businesses and everyday tasks. Attendees will garner tips and share ideas on how we make these initiatives sustainable (innovative educational projects, incentives), not lose focus or get bored of the topic, establish baselines in a food safety culture's understanding/level of engagement of food safety, and discover ways to measure/show progress?

We will be asking our final speaker to use the interactive Alchemy remote system to conduct a short quiz for the audience, during his presentation.

S41 A Physical Firewall: Packaging for Food Protection

JOSEPH MARCY: *Virginia Tech, Blacksburg, VA, USA*
JIM BELCHER: *Cryovac Division of Sealed Air, Duncan, SC, USA*
ALAN CAMPBELL: *Campden BRI, Chipping Campden, United Kingdom*

The world food packaging market is worth over $200 billion, with businesses in North America representing the largest customer base, accounting for 31% of annual sales. Polymer materials represent 30% of this food packaging market sourcing materials globally. How do we know all this food packaging is safe and how the packaging contributes to the safety of the food itself?

In the 21st century, the growth in food packaging is being driven by the increased use of packaged food. There's a shift to central packaging operations, along with a rising demand for ready-prepared, convenience foods, including microwave meals for time-pressed consumers. Other key trends include the rise in smaller households, leading to increasing units of smaller pack sizes, portion control, as well as the shift from metal and glass to plastic packaging to flexible materials for items like soups, sauces, pet food and other products.

This trend of conversion to plastic materials, convenience and sustainability coupled with extended shelf life drives the food manufacturer to source cost effective packaging solutions globally to meet the needs of the consumer. Given these developments, how do we ensure the polymers are effective barriers while achieving the highest safety standards? At the same time, how can we package our food in an eco-friendly, sustainable manner using the cradle to cradle concept?

This symposium will showcase the key global challenges within the food packaging polymer sector and how they're being addressed by stakeholders. Expert speakers who are actively engaged with food packaging from cradle to cradle will cover 1) food packaging safety issues and collaborative solutions for best practices (meat and dairy sectors), 2) practical solutions for achieving the ultimate integral food package to ensure food safety (meat sector) and 3) global food packaging trends and safety testing requirements.

S42 What in the World is Going on with My High-acid Aseptically Packed Product? An International Perspective of Challenges and Benefits

L. JASON RICHARDSON: *The Coca-Cola Co., Atlanta, GA, USA*
WILFREDO OCASIO: *The National Food Laboratory, Inc., Livermore, CA, USA*
SEAN LEIGHTON: *The Coca-Cola Company, Atlanta, GA, USA*
PAUL GERHARDT: *The National Food Lab, Livermore, CA, USA*
MARGO PIDGEON: *PepsiCo, Chicago, IL, USA*
DAVID MONDIEK: *Abbott, Cleveland, OH, USA*

Much effort and research has been placed on the validation and microbiological safe operations of low-acid, shelf-stable systems throughout the world. Comparatively little information and guidance is available for their high-acid counterparts mainly due to the misconception that the high acidity precludes microbiological issues. However, these products face many microbiological challenges such as acid tolerance of vegetative bacterial pathogens and high heat resistance of aciduric spoilage organisms such as heat-resistant molds (HRM's) and bacterial spores (e.g., Alicyclobacillus and Sporolactobacillus). Aseptically filled products can also be contaminated by lactic acid bacteria, acetic acid bacteria, and non-heat-resistant yeasts and molds. In addition to the microbiological challenges, global companies must deal with the complexities and diversity of regulatory requirements (including FSMA) and industry standards used throughout the world. Additionally, large complex fillers such as high speed rotary aseptic fillers present further challenges due to their size and complexity.

This symposium aims to provide expert perspective on the wide diversity of high-acid (pH < 4.6) products packaged aseptically around the world and the regulatory, technical and microbiologically challenges they face. While formulated beverages and fruit juices dominate this category, other products such as baby food, fruit purees and desserts are also packaged aseptically. The symposium will cover aspects related to aseptically filled high-acid products including microorganisms of significance, strategies to prevent microbiological contamination, microbiological safety validation procedures and criteria, regulatory requirements in the USA and abroad, identifying, selecting and qualifying contract manufacturers, and new technologies for aseptic processing and packaging.

S43 Virus Detection in Foods: Advancements and Applications

JENNIFER CANNON: *University of Georgia, Griffin, GA, USA*
ANGUS KNIGHT: *Leatherhead Food Research Lab, Leatherhead, United Kingdom*
MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium*
DALLAS HOOVER: *University of Delaware, Newark, DE, USA*
TOM ROSS: *University of Tasmania, Hobart, Australia*
SOPHIE BUTOT: *Nestle, Lausanne, Switzerland*

Enteric viruses, particularly human noroviruses (NoV) are the most common cause of food borne disease, responsible for up to 50% of all outbreaks and cases per year in the U.S. Viruses enter the food supply across the farm-to-fork chain by exposure to contaminated waters, surfaces, and/or human hands. Unlike bacterial pathogens, for which there are widely used validated detection technologies, virus detection methods are less well developed. There are many reasons for this which, when taken together, necessitate processing food or environmental samples for virus concentration and purification prior to detection using molecular-based methods. This process is cumbersome, expensive, inefficient, and fraught with complications, including the inability to discriminate infectious from non-infectious viruses. Many laboratories are attempting to both improve and use detection technologies to better understand the transmission and control of viruses in the food chain. The purpose of this symposium is to discuss recent developments in virus detection strategies, and how these detection methods can be used to address real-world issues for the food industry and regulators. The first part of the symposium will cover scientific advances that are making virus detection more practical and reliable. In the second portion of the symposium, speakers will discuss how key considerations in food processing, sampling, and testing impact the utility of virus testing. The symposium participant should come away with an increased understanding of the complexities associated with detection of viruses in foods and environmental samples, and how these complexities are being addressed so that we can more routinely use virus detection to protect the food supply and public health.

S44 Recent Developments in Norovirus Research

JASON JIANG: *Cincinnati Children's Hospital, Cincinnati, OH, USA*
ABIMBOLA KOLAWOLE: *University of Michigan, Ann Arbor, MI, USA*
JUAN LEON: *Emory University, Atlanta, GA, USA*
MELISSA HERBST-KRALOVETZ: *University of Arizona, Phoenix, AZ, USA*

Human norovirus is now recognized as the major cause of foodborne illness in many parts of the world. While substantial research has been performed using surrogate viruses, it is increasingly clear that the utility of surrogates for answering key questions about human norovirus is limited. Currently many basic questions about human norovirus are largely unknown since the virus cannot be replicated in the laboratory.

In this symposium proposal, we intend to present forward-looking research on norovirus, which will be critical in our ability to control spread the pathogen. For example, research on *in vitro* replication of human norovirus focusing on stems cells appears promising; could this be a long-awaited break through? One way to determine infectivity of human norovirus is through human volunteers trials; how feasible and safe are they? Research into norovirus vaccine development is progressing; will it someday be possible to vaccinate against norovirus?

The focus on state of the art research will provide the audience a rounded understanding of the challenges faced by researchers, as well as the promising avenues that are being explored to address the public health concern around human norovirus.

S45 Food Allergen Thresholds and Food Labeling: Challenges and Perspectives

STEVE TAYLOR: *University of Nebraska-Lincoln, Lincoln, NE, USA*
STEVEN GENDEL: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
MARK MOORMAN: *Kellogg Company, Battle Creek, MI, USA*
PATRICE ARBAULT: *BioAdvantage Consulting, Orlienas, France*

Food labeling regulations have been published by regulatory agencies in Europe, the U.S. and Canada that include specifications for how to disclosure of the presence of food allergens. At the same time, the U.S. FDA and the EU DG/SANCO have defined levels of gluten that qualify for the use of "gluten free" labeling. In addition to government regulations, the industry VITAL program in Australia has proposed thresholds for various food allergens and is working to expand the program to other countries. This symposium will consider the use of these thresholds and issues related to implementation such as the application of current detection methods, the needs and expectations of consumers, and pending questions for industry.

S46 Celebrating 100 Years of Food Safety

FRANK BRYAN: *Food Safety Consultation & Training, Lithonia, GA, USA*
EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*
WILLIAM SPERBER: *The Friendly Microbiologist, LLC, Minnetonka, MN, USA*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
ERNEST JULIAN: *Rhode Island Department of Health, Providence, RI, USA*

The Committee for Control of Foodborne Illness has been in existence for 100 years, preceding the formation of the International Association of Milk, Food and Environmental Sanitarians. The purpose of this symposium is to review the history of foodborne disease and its control, with the changes that have led to improvements.

How much our awareness of pathogens has changed over time together with the enormous increase in the global food supply available to the consumer today will be discussed. This has extended the need for traceability of food ingredients and products across continents as well as in the local community. New epidemiological and molecular typing has made it possible to detect small numbers of widely spread infected individuals to be aware of multi-state and multinational outbreaks. Industry has cooperated with government to use more prevention and control strategies and international standards. Some feel that government oversight is excessive and others feel it has not reduced the risk of foodborne disease adequately. However, today most foodborne illnesses still occur at the local community as they did 100 years ago, and the burden of prevention and control rests with the local food operation and environmental health officer. We also recognize that food safety needs to be cooperative and not competitive among those producing and overseeing food processing and service. The speakers will review the history but what we have learned over the 100 years and look forward to further improvements for food safety, and what roles to scientific societies like IAFP have to contribute. The suggested speakers reflect long-term association with issues around food safety but there are many other competent speakers that could be asked.

S47 Threshold of Toxicological Concern (TTC) — A Pragmatic Approach to Determine the Risk of a Chemical Substance of Unknown Toxicity

MITCH CHEESEMAN: *Steptoe & Johnson LLP, Washington, D.C., USA*
SEAN TAYLOR: *International Organization of the Flavor Industry, Washington, D.C., USA*
RICHARD CANADY: *ILSI Research Foundation, Washington, D.C., USA*
PENELOPE RICE: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Continued advances in analytical chemistry techniques lead to greater discovery and detection of very low levels of chemical substances in food. The spectrum and sheer magnitude of low level chemical constituents in the diet presents a challenge to assessing safety. The Threshold of Toxicological Concern (TTC) is a principle that refers to the establishment of a generic human exposure threshold value for groups of chemicals below which there would be a low probability of risk to human health. The concept proposes that such a value can be identified for many chemicals, including those of unknown toxicity based upon their chemical structures. The TTC approach is beneficial to consumers, industry, and regulators by prioritizing necessary testing and extensive safety evaluations to instances when exposure is above a relevant threshold value. This session will review the TTC tool by providing the scientific basis and history of TTC, its applicability to chemical substances found in food and use of TTC by regulatory authorities.

S48 Food Safety Challenges and Implementation of Food Safety Regulations in Indian Subcontinent

PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
DON ZINK: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
AJIT KUMAR: *National Institute of Food Technology Entrepreneurship and Management, Kundli, India*
DEEPA BHAJEKAR: *Silliker, Inc., Mahape, India*
NILESH AMRITKAR: *Envirocare Labs, Thane, India*

Rapid globalization of commerce and pursuant movement of food worth about one trillion US dollars in the last two decades called for more scrutiny of the safety of the safety of foods. The Indian subcontinent has recently emerged as one of the players in the global commerce including food sector business. However, only in recent times, the subcontinent started placing emphasis on food safety. For example, India has passed a comprehensive food safety law in 2006 creating Food Safety and Standards Authority of India (FSSAI) which came up with regulations for safety and standards of food in August of 2011. Other countries like Pakistan also have instituted food safety laws in Punjab region. FSSAI is actively involved in implementing the food safety standards, and traceability regulations in the midst of many challenges ranging from risk analysis to monitoring and surveillance. A good illustration is the developing and implementing regulations for street food vendors vis-à-vis legislation for protecting the livelihood of the street vendors and court litigations. Likewise, registration and licensing of millions of these facilities is a challenge. Another example of challenge in India is outreach activity which has not reached the food processing industry. The subcontinent also faces another challenge of producing food that is meant for export. While many countries, including the subcontinent, are confused about how US FDA will come with a viable foreign supply verification and third party certification programs of the Food Safety Modernization Act of U.S. (and equivalents of other developed countries). In this symposium, both internal and external challenges will be discussed in detail.

S49 Equipment and Facility Contributions in Cases of Foodborne Illness Outbreaks and Recalls

STEVEN SIMS: *U.S. Food and Drug Administration, College Park, MD, USA*
DON ZINK: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
CAROL SELMAN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
RUTH PETRAN: *Ecolab Inc., Eagan, MN, USA*

There are many things we can learn from the recent foodborne illness outbreaks and recalls. If we step back and look at the equipment used in the production of food products, can it be cleaned and sanitized? Did the food plant design and construction contribute to product contamination? Microbes are hitchhikers and need a means of transfer from one source to another. How has this played into these outbreaks and recalls? A used piece of equipment a processor purchased to enhance food safety of their product contributed to illnesses and deaths. In other cases the equipment could not be taken apart for cleaning and sanitizing. The equipment became a distributor for microbial contamination. Ledges and niches inside of equipment can provide the hiding palace for microbes and allergens, awaiting the opportunity to cause a product recall. The most common regulatory demerit observed is related to facility maintenance and construction connected to product protection. Buckets, plastic film and duct tape are not the answer to roof leaks. There have been several recalls related to roof leaks where leakage provided a ride for salmonella into the plant so the employee shoes could distribute it around the plant. Cleaners and sanitizers are wonderful materials for use in providing a clean sanitary environment for food production. If that food contact surface and surrounding areas are not accessible or have surfaces that cannot be reached due to design problems, the best of cleaning materials cannot prevent a problem. If you cannot see it, you cannot clean it. Cleaning and sanitizing is a reoccurring cost over the life of equipment/facility and any improvements in the ability to properly clean and sanitize is an investment with a pay back in Brand protection and labor savings.

S50 Pathogenic Lethality Characteristics of Cheese Made from Unpasteurized Milk

DENNIS D'AMICO: *University of Connecticut, Storrs, CT, USA*
DEAN SOMMER: *University of Wisconsin-Madison, Madison, WI, USA*
OLIVIER CERF: *École vétérinaire d'Alfort, Maisons-Alfort, France*
OBIANUJU NSOFOR: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

The U.S., Canadian and European regulatory systems, as well as many other governments around the world have relied on "60-day" aging as a measure to achieve an acceptable level of safety for cheese made from unpasteurized milk. This measure is based on scientific studies completed decades ago using now outdated and much less sensitive pathogen detection techniques. This "safe harbor benchmark" needs to be revisited in light of more recent scientific studies, pathogenic detection technology and a better understanding of the chemical dynamics of cheese aging. This symposium brings together experts in the areas of cheese & dairy chemistry, cheese processing, cheese aging, and federal dairy safety regulations to provide the most current understanding and science in an attempt to evaluate if "60-day" aging really delivers an acceptable level of safety and if it does not, what are some new "safe harbor benchmarks" that could be used.

With a large percentage of the North & South American as well as European population consuming large amounts of cheese, this symposium is a must attend for anyone involved in the cheese processing industry or regulating this industry, particularly for those having to deal with raw or heat-treated milk as their main ingredient.

S51 High Pressure Processing – State of the Science and the Art of Application

KEVIN MYERS: *Hormel Foods Corporation, Austin, MN, USA*
JAMES DICKSON: *Iowa State University, Ames, IA, USA*
LYNN MCMULLEN: *University of Alberta, Edmonton, AB, Canada*
DAVID KINGSLEY: *U.S. Department of Agriculture-ARS, Dover, DE, USA*
CATHY MOIR: *Commonwealth Scientific and Industrial Research Organisation, Melbourne, Australia*

High Hydrostatic Pressure, also known as High Pressure Processing (HPP), is an increasingly popular technology by which food products are subjected to isostatic pressure in the range of about 100 to 690 MPa, usually while within enclosed final packaging and at refrigeration or ambient temperature. Numerous research studies report the efficacy of HPP against a variety of bacterial, parasitic, and viral pathogens in a variety of food systems including raw and cooked meat, poultry and seafood, fresh and processed produce, acid and acidified foods and beverages, and low-acid refrigerated foods. HPP can also be effective against spoilage microorganisms and extend shelf life of perishable foods. Depending on application, HPP is typically used in lieu of traditional thermal processing or of preservatives. Experts on HPP research, validation, and implementation will present recent findings concerning the efficacy of HPP against pathogens and spoilage microorganisms. The state of the science will be covered along with lessons learned from applying HPP to different food systems.

An overview of HPP will be provided by an industry leader in large-scale use of HPP to process and preserve meat products. This will segue into a presentation on recent HPP research for control of *Listeria monocytogenes* in RTE meat products without added nitrates or nitrites. Afterwards, research will be presented concerning unexpected efficacy of HPP against certain sporeforming bacteria of importance to processed meat and other food systems. The next presentation will transition to use of HPP in meat and non-meat systems, particularly in the Asia Pacific markets. Then, an overview of the efficacy of HPP against viral pathogens or their surrogates in seafood, fresh produce, and acid or acidified foods and beverages will be presented. Finally, the session will conclude with a presentation on the HPP-inactivation of fungal spores in high acid beverages.

Roundtable Abstracts

RT1 Peer-reviewed Publishing in Food Safety: It Doesn't Have to be *That* Painful

LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
ELLIOT RYSER: *Michigan State University, East Lansing, MI, USA*
LAURA STRAWN: *Cornell University, Ithaca, NY, USA*
JESSIE VIPHAM: *Texas Tech University, Lubbock, TX, USA*

Graduate students rather often have a hard time planning, writing and submitting scientific manuscripts for peer-reviewed publication. For some, publishing can become a torturous process, especially for scientists that do not have very developed writing skills, and many postpone writing until the last moment. The Student Professional Development Group (SPDG) firmly believes that developing effective writing skills is an essential part of becoming a successful food safety professional. Consequently, the focal point of this roundtable will be to provide the student community advice on how to face the process of peer-reviewed publishing, from conception of the manuscript to addressing reviewers' comments. Highly published faculty members and journal editors in the field of food safety will give the audience their perspective on how to make the road to successful publishing less painful and more effective. Students will be made aware of current issues in scientific publishing, including the selection of an audience and the definition of authorship. Ultimately, these experts will help us understand what constitutes a manuscript suitable for publication.

RT2 Establishing Science-based Performance Standards: Are We There Yet?

MARGARITA GOMEZ: *Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA, USA*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration/IFSH, Bedford Park, IL, USA*
MICKEY PARISH: *U.S. Food and Drug Administration-CFSAN, Silver Spring, MD, USA*
NICHOLAS ASHBOLT: *University of Alberta, Edmonton, AB, Canada*
WILFREDO OCASIO: *The National Food Laboratory, Inc., Livermore, CA, USA*

The food industry has relied on generally recognized safe harbors in manufacturing to assure delivery of safe foods. These provide a 'blanket' framework for defining performance standards, and have been established over time by industry consensus or regulation. They are typically expressed as the number of log-reductions of a hazard of concern (e.g., 12-log reduction of proteolytic *Clostridium botulinum*in low acid ambient stable foods or 5-log pathogen reduction for juices). Although such an approach is convenient as it allows for establishment of process/product criteria without detailed knowledge of the level of raw material contamination or the variability of the process, it may lead to misinterpretation of what the performance standard actually means and what it does in terms of assuring consumer protection. The use of 'end-point' targets to define the safe establishment of performance standards is commonly used in other industry sectors. In the drinking water area, the World Health Organization has established health-based targets based on a tolerable burden of disease, and derived performance standards for household treatment options derived from such targets. However, in the food industry, in spite of the existence, for more than a decade, of well-defined 'endpoint' risk management metrics such as Food Safety Objectives (FSO) or Performance Objectives (PO), the establishment of science-based performance standards has not been fully realized yet. The definition of science-based performance standards based on metrics such as FSO/PO requires cooperation between key stakeholders, e.g., industry, regulators, researchers, and key opinion formers.

This roundtable will bring together relevant stakeholders to address these issues and to discuss what is needed to realize the establishment of 'end-point' targets to define science-based performance standards.

RT3 Food Safety or Food Availability: Do We Have to Choose?

MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
CRAIG HEDBERG: *University of Minnesota, Minneapolis, MN, USA*
SHAUN KENNEDY: *University of Minnesota, St. Paul, MN, USA*
ATINA DIFFLEY: *Organic Farming Works LLC, Farmington, MN, USA*

Increasing access to healthy food has become a key focus area of efforts to reduce chronic diseases and to end hunger and food insecurity. The efforts have largely focused on improving the availability and affordability of whole grains, fruits, vegetables, and lean protein sources. Efforts to promote access to healthy food can be the source of concerns about safety surrounding how food is grown and handled. Opportunities for intentional and/or unintentional breaches in the food system, which may put the public at risk and hinder market access, could be created. Food safety measures are likely to increase food costs and may decrease the production of some crops due to risks. What is an appropriate level of protection (ALOP), and is it necessary to reconcile differences in standards based on the wealth of nations and within nations? How will proposed regulation impact safety and international trade of nutritionally important crops? Can we take lessons from environmental protection standards and effects on competitiveness of industries in a global market, or will the focus be on an individual country basis? With limited resources, should small producers be the focus of any outreach efforts when the relative risk to the population is low? As demand for increased food safety is happening as the world's population is expanding, are there choices to be made for seeking a balance between food safety and food availability?

RT4 Food Dating Confusion – Solutions for Public Health and Social Responsibility

KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
CAROLINE SMITH DEWAAL: *Center for Science in the Public Interest, Washington, D.C., USA*
FRANK YIANNAS: *Walmart, Bentonville, AR, USA*
HILARY THESMAR: *Food Marketing Institute, Arlington, VA, USA*
JENNY SCOTT: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
EMILY BROAD LEIB: *Harvard Law School Center for Health Law and Policy Innovation, Jamaica Plain, MA, USA*

Food date labels are inconsistently regulated by states, and are not clearly defined or applied, even in the same product categories. In the majority of cases, the date labels don't actually indicate when food will spoil, or provide a reliable measure for managing food safety, but are an indicator of optimal quality determined by the manufacturer. This leaves consumers confused.

A recent study by the Harvard University Law School and the Natural Resources Defense Council reported that Americans throw away 40 percent of the food they buy often because of misleading expiration dates that may not be food safety specific. The current date labels may impart a false sense of food safety if the end user simply makes decisions based on the label dates. While in reality, critical food safety factors or controls (i.e., water activity, pH) are different for different food types. For example, the factor for an end user to judge the food safety of a package of ready-to-eat deli meat is not only the total duration of storage, but also the amount of time the food has spent in the "danger zone". Our food dating and end user communication systems should be re-assessed by specific food type.

A report by the Food Marketing Institute indicated that 91 percent of consumers disposed of food after the sell-by date at least occasionally, and a whopping 25 percent *always* did so. The resulting food waste must be diverted which contributes complexity and potential risk to public health.
The purpose of this roundtable will be to discuss the current food labeling systems and provide insight into more effective science-based alternative solutions that decrease confusion and food waste while enhancing food safety and public health.

RT5 HACCP – Clear as Mud

DAVID ACHESON: *Leavitt Partners, Glenelg, MD, USA*
SARA MORTIMORE: *Land O' Lakes, Inc., St. Paul, MN, USA*
ROBERT GAZE: *Campden BRI, Chipping Campden, United Kingdom*
GILLIAN KELLEHER: *Wegmans Food Markets, Inc., Rochester, NY, USA*

Objective of this roundtable is to engage the participants in a structured discussion on the topic of HACCP utilization in an ever-changing environment. Each speaker will be posed two central discussion points ahead of the meeting and the discussion will be moderated to foster audience participation. Speakers will discuss topics of regulated HACCP (i.e., from a CFIA and FDA perspective), changing HACCP requirements (i.e., GFSI and benchmarked schemes) and the potential weakness and conflicts between regulations and GFSI recognized schemes, in fundamentals to support both. Questions: 1) In an ever-changing industrial world, which do you think is a higher priority for ensuring the success of HACCP systems: Innovation or adherence to historical doctrine?, 2) With all of the different forms of HACCP available to food manufacturers today, what are the potential weaknesses and/or conflicts between regulations and other recognized schemes?

RT6 Threats *vs.* Opportunities for Food Safety and Public Health Surveillance through Culture Independent Methods

MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
WENDY WARREN: *AEGIS Food Testing Laboratories, North Sioux City, SD, USA*
EMILIO ESTEBAN: *U.S. Department of Agriculture-FSIS-OPHS-EALS, Athens, GA, USA*
J. STAN BAILEY: *bioMérieux, Athens, GA, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration, College Park, MD, USA*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*

A mini-symposium on "Culture Independent Diagnostics" was organized during the IAFP 2013 Annual Meeting. The topic is of utmost interest to the food safety stakeholders as culture independent methods are emerging for microbial diagnostic applications in food. These methods do not require cultural isolation of pathogens and thus are unconventional for routine testing in food microbiology laboratories. This follow-up roundtable is organized to further discuss the dilemma that lies among the different stakeholders on various threats vs. opportunities for food safety and public health surveillance through these culture independent methods. Panelists for the roundtable representing stakeholders in food safety including the USDA and USFDA would discuss various concerns such as validation and verification requirements, routine laboratory testing including those requiring cultural isolates, outbreak investigation, public health surveillance and management, and global information sharing, etc. Representative questions that will be addressed during the discussion are:

- What is the scope of culture-independent methods? How does it differ among different stake-holders?
- What are key opportunities for food industry and public health agencies when using culture independent methods over the existing microbiological methods?
- What are key challenges when using culture independent methods over the existing microbiological methods?
- How will public health surveillance be performed including foodborne outbreak events if culture independent methods are applied?

RT7 Food Safety Consulting: A Roundtable

JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
MELISSA CALICCHIA: *Food Microbiological Labs, Cypress, CA, USA*
PAULA PIONTEK: *red24assist, Milwaukee, WI, USA*
PATRICE ARBAULT: *BioAdvantage Consulting, Orlienas, France*
DAVID BLOMQUIST: *Ecolab, Eagan, MN, USA*

Food safety consultants are employed in a broad range of venues, from large corporations (e.g., Ecolab, Eurofins, Silliker, Food Safety Net, IEH, etc.), to smaller companies, to self-employed individuals. Much of their work is on the front lines of food safety investigations, recalls and foodborne illness outbreaks. It has been said, "learn from the mistakes of others; you don't have enough time to make them all yourself." Learning from the experience of food safety consultants could assist food safety professionals in preventing future cross-contamination issues, product recalls and foodborne illness outbreaks. In a world approaching 9–10 billion by mid-century, the demand for food safety consultants will grow commensurately. This roundtable will help other consultants and food safety experts prepare for heretofore unforeseen challenges, and even inform students or mid-career employees as to the feasibility of entering this dynamic field. The five confirmed participants will answer a series of pre-assigned questions in the first half of the session, and respond to audience-generated questions during the second half.

RT8 As the World Turns: A 360 Point – Counterpoint of Global Issues Related to Food Animal Production and Antimicrobial Resistance

CAROLINE SMITH DEWAAL: *Center for Science in the Public Interest, Washington, D.C., USA*
AWA AIDARA-KANE: *WHO, Geneva, Switzerland*
JENNIFER KOEMAN: *National Pork Board, Clive, IA, USA*
JEFFREY LEJEUNE: *The Ohio State University, Wooster, OH, USA*
ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Symposia afford attendees the opportunity to hear the latest information on a specific topic with little time for questions. For emerging and controversial topics it affords little or no time for adequate question and answer. The purpose of this roundtable is to facilitate a 360 global view on one of the most passionately debated issues – developing antimicrobial resistance in bacteria associated with food animal production. We will invite a scientist, veterinarian, physician, commodity group representative, lawyer and a WHO expert representing developing countries to provide frank debate, discussion and opposing opinions on antimicrobial use in food animal production, emerging resistance attributes of concern, feeding global populations and food production when the population reaches 10 billion people (projected in 2050) utilizing the same land mass as today while juggling animal welfare, housing and the food supply, food borne outbreaks, reporting in the media, and controlling emerging resistance as the world becomes an infinitely smaller place particularly as agriculture and man come in closer and closer contact through agricultural practice, housing and travel. The audience will be asked to participate by voting on responses (for instance – as highly likely to succeed in implementation) as well as submitting questions for the moderators to present. Moderators will be prepared to start the questions and envision alternating an audience question into the mix to keep the discussion flowing and participation high. Moderators will ensure the questions stay balanced and fair and the discussion remains productive. This roundtable will serve as a unique model for future open and frank discussion of sensitive topics.

RT9 Debate - Current Perspectives in Food Safety

ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
TIM FREIER: *Cargill, Inc., Minneapolis, MN, USA*
TIM JACKSON: *Nestle USA, Inc., Glendale, CA, USA*
ROBERT BRACKETT: *Illinois Institute of Technology, Bedford Park, IL, USA*
BARBARA KOWALCYK: *Center for Foodborne Illness, Raleigh, NC, USA*
MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*

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 symposium. The session will cover three topics: "Risk of illness from raw sprout consumption cannot be adequately controlled"; "Effective HACCP programs should negate the need for routine finished product testing yet customer requests for such testing are increasing"; and "Recalls are not an effective tool in the food safety tool box." Each topic will include a 9-min presentation in support of (PRO) followed by a 9-min presentation in opposition to (CON) the proposed question. Each speaker will prepare a minimum of 2–3 slides. Each speaker will have 3-min for extemporaneous rebuttals. A 6-min question/answer session will then follow to allow for audience participation. We will have electronic voter buttons for the audience to use to vote Yes/No/Undecided. A vote is taken at the beginning and again at the end to see whether people's views have changed. 1) Risk of illness from raw sprout consumption cannot be adequately controlled. Pro: Michelle Smith, FDA/CFSAN; Con; Robert Tauxe, CDC 2) Effective HACCP programs should negate the need for routine finished product testing yet customer requests for such testing are increasing. Pro: Tim Freier, Cargill, Incorporated; Con: Tim Jackson, Nestlé USA, Inc. 3) Recalls are not an effective tool in the food safety tool box. Pro: Robert Brackett, Institute for Food Safety and Health, Illinois Institute of Technology; Con: Barbara Kowalcyk, Center for Foodborne Illness Research & Prevention.

RT10 Managing Norovirus at Retail: What's the Food Safety Manager to Do?

MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*
ANNA STAROBIN: *Ecolab, Greensboro, NC, USA*
KEVIN SMITH: *U.S. Food and Drug Administration, Washington, D.C., USA*
JAMES ARBOGAST: *GOJO Industries, Inc., Akron, OH, USA*
JOHN TILDEN: *Michigan Department of Ag. and Rural Development, Lansing, MI, USA*

Human noroviruses (HuNoV) are the leading cause of foodborne illness in the U.S. and probably in many other countries. Virus contamination of foods frequently occurs as a consequence of poor personal hygiene practices of infected food handlers. Epidemiological and laboratory-based evidence also confirm that HuNoV are released and aerosolized during projectile vomiting incidents, the hallmark symptom of disease. Foods prepared and consumed away from the home (retail, grocery, and institutional settings) are at particular risk for contamination. Unfortunately, HuNoV are notoriously recalcitrant to most disinfectants and sanitizers when used at manufacturer-recommended concentrations. This presents a quandary for the retail and institutional foods sector, specifically: "How does one effectively control virus contamination of foods served in their establishments without unduly alarming customers and within the mandates of existing regulations." The purpose of this roundtable session is to have a frank discussion of this issue. Some key points of discussion will include the following, with a specific focus on the retail and institutional sectors: (i) examples in which HuNoV have been transmitted via contaminated foods at retail; (ii) how foods become contaminated with viruses at retail; (iii) the most likely reservoirs for virus contamination; (iv) commonly used disinfection practices; (v) how to manage on-site vomiting events; (vi) what is the role of the Food Code in addressing the HuNoV problem; and (vii) future research and practical needs to control foodborne virus contamination in association with retail and institutionally prepared foods. Representatives from the public health, retail/institutional, and sanitizer/disinfection sectors will participate as members of the roundtable. The participants will learn more about the problem, what is being done, and what needs to be done to practically and effectively control foodborne viruses in these key food sectors.

RT11 IFIC Communications Roundtable: Leveraging Your Networks to Enhance Food Risk Communications

JANE ANDREWS: *Wegmans Food Markets, Inc., Rochester, NY, USA*
MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*
CHRISTINE BRUHN: *University of California-Davis, Davis, CA, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
WILLIAM HALLMAN: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
MATT RAYMOND: *International Food Information Council and Foundation, Washington, D.C., USA*
JEFF SIMMONS: *Elanco, Greenfield, IN, USA*

Many professionals are frustrated by today's media environment in which food safety, food technology and other food related issues are often miscommunicated, causing undue public concern--especially for the mothers of young children. Many observers want to help or engage, but don't want to be on their own or don't know where to start. An effective food risk communication and education strategy is necessary to put food risks and benefits into the proper perspective for the consumer. There is an opportunity to leverage each of our various networks at home, at work, in our faiths, with our friends and many others to become catalysts and conveyers of the best information on food and health, delivered in a compelling way. Learn about a new network organized by the International Food Information Council Foundation which seeks to do just that. This panel of science and communication

experts will provide case studies and a range of perspectives to expand the conversation beyond traditional risk communication to empower a grass-roots network to balance the public dialogue on food and health.

RT12 Improving Public Understanding from Farm to Table: Let's Talk about Food Safety and Food Biotechnology

JANE ANDREWS: *Wegmans Food Markets, Inc., Rochester, NY, USA*
JOHN VICINI: *Monsanto, St. Louis, MO, USA*
MARIANNE SMITH EDGE: *International Food Information Council, Washington, D.C., USA*
STEVEN GENDEL: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
RICK GOODMAN: *University of Nebraska-Lincoln, Lincoln, NE, USA*
ROBERT THOMPSON: *Johns Hopkins School of Advanced International Studies, Washington, D.C., USA*

Consumer perceptions about food safety continues to evolve. While consumer confidence remains high and foodborne illness remains a top food safety concern for consumers, it's important to know today's media and social environments transmit information that could potentially impact consumer perceptions about food safety – for better or worse. In addition to confidence and concerns about food safety, consumer interest about the origins of food has increased in recent years, due to many being further removed from the farm, as well as greater attention given to food and our health. As a result, media coverage of these issues, including the use of modern food production and agricultural technologies, has increased. However, the beneficial roles that food technology plays in improving food safety, sustainability and our ability to feed a growing population often gets lost in translation before it ever reaches the consumer. So what do consumers think about food safety? What do consumers think about food technology? Have their perceptions about these topics changed in light of negative media coverage? What are common misperceptions about food safety and technology that are being perpetuated in the media? What and where are the opportunities to provide more balanced, science-based information on these topics? This roundtable discussion will address these questions with a dual emphasis on food safety and food biotechnology. The roundtable experts will provide unique perspectives from the various segments of the food supply chain – retail, industry, consumer, government, and academia – and discuss consumer perceptions about food safety as well as explore the benefits and perceived risks associated with modern food production. Roundtable participants will share their successful interaction efforts with the media ultimately educating consumers about their role in creating a more sustainable and safe food supply.

RT13 The Changing World of Scientific Publication

MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*
DONALD SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
AMANDA FERGUSON: *Institute for Food Technologists, Chicago, IL, USA*
BYRON CHAVES: *Texas Tech University, Lubbock, TX, USA*
P. MICHAEL DAVIDSON: *University of Tennessee-Knoxville, Knoxville, TN, USA*

In the world of science, one of the most important components is the ability of the scientists to publish their data and get credit for these in their career path, whether in academia, government or industry. There are significant developments that challenge both journals and potential authors. These include traditional time-delayed but reader-paid subscriptions vs. authors paying for real-time publications; plagiarism from other authors or self plagiarism, and the role of plagiarism-detection software like iThenticate; more detection of deliberate or careless scientific fraud through pressure to get more publications for tenure/promotion; the peer review process – is it working well enough; proliferation of new journals, often with genuine sounding titles reflecting long-term publication history but in fact are brand new, with increased marketing for submissions (also for conference abstracts and attendance). Scientific societies have to consider how to compete in this world of a plethora of competing journals, some of which can be called rogue in that they are seeking economic gain over scientific excellence. Researchers have to weigh options of where to publish and what the long-term effect will be on their careers. Are the traditional monthly hard copy journal issues a thing of the past as more real-time electronic papers are becoming the norm? This roundtable will have panelists representing four scientific journals, IAFP, a mature student perspective to review the different issues and suggest ways of pathways out of the publication maze.

RT14 Economic Impact and Corresponding Public Health Benefit of FSMA Proposed Regulations

STEVE MAVITY: *Bumble Bee Foods, Chicago, IL, USA*
TIM JACKSON: *Nestle USA, Inc., Glendale, CA, USA*
RICHARD WILLIAMS: *George Mason University, Arlington, VA, USA*

Now that the Food Safety Modernization Act (FSMA) has been upon us for more than three and a half years, what will be the economic impact to the food industry along with the corresponding public health benefit? This roundtable discussion capitalizes on industry's calculated costs to implement FSMA, as written, in the proposed rules for Preventative Controls for Human Food, Focused Mitigation Strategies to Protect Food Against Intentional Adulteration and Preventive Controls for Food for Animals. Hear industry's key concerns and how the economics of the food manufacturing industry could be impacted. Understand the Agency's calculations included in the Preliminary Regulatory Impact Analysis (PRIA) in comparison to industry's calculations. Audience members will have the opportunity to pose questions to each panelist regarding the specifics of the economic impact and the associated public health benefits along with the general approach of the PRIAs. Key challenge areas are encouraged to be discussed.

RT15 Public and Private Food Safety Research Funding: Where Should the Money Go?

DAVID ACHESON: *The Acheson Group, Glenelg, MD, USA*
MICHAEL DOYLE: *University of Georgia, Griffin, GA, USA*
JEANETTE THURSTON: *U.S. Department of Agriculture-NIFA, Washington, D.C., USA*
CAROLINE SMITH DEWAAL: *Center for Science in the Public Interest, Washington, D.C., USA*
JOAN MENKE-SCHAENZER: *Conagra, Inc., Omaha, NE, USA*

Hundreds of millions of public and private dollars fund basic and applied food safety research, from organizations such as NIFA, ILSI, AMI, CPS, U.S. Poult. & Egg Assoc., Bill and Melinda Gates Foundation, other federal and local governments around the world. In addition, private-funded, industry-related projects abound. Many of these grants are driven not only by the "hot topics" of the day, and issues perceived as the greatest threat to public health, but also stem from problems representing the greatest economic losses to the food industry (which may or may not be significant food safety issues). While all these areas are important, the question has been posed: *"are grant-funded research dollars being allocated and utilized in the maximally effective way to improve food safety?"* Participants of this roundtable will represent various areas of food production, regulation, research, and consumer interests, and will be able to provide point-counterpoint arguments relative to food safety research funding. Topics for discussion will include (1) Maximally beneficial versus less impactful uses of research dollars, (2) Comparison of the food safety impact between [a] publishable research and public access data versus [b] proprietary research projects, (3) Human illness-driven versus industry economic loss-driven research, (4) The effects of pseudo-science non-food safety activism on impactful research (e.g., "pink slime" and genetically engineered crops), (5) The practicality of surrogate microorganisms versus pathogenic microorganisms in research, (6) The use of human or animal models versus cell culture or other *in vitro* techniques, (7) Challenge studies versus mathematical modeling, and (8) Lab-scale versus manufacturing plant or agricultural field trial studies.
The following questions will be pre-assigned for speakers to consider:

1. Are current research dollars being used to the maximum extent for food safety? If not, how could funding allocations be improved?
2. Where does the balance lie in funding research projects that are "human-illness driven" versus projects that stem from "food industry profit losses?"
3. Discuss the impact of research publication and data sharing versus proprietary research, which benefits only a single producer or group of producers. (i.e., What is the impact of competition among food producers such that important food safety information remains in-house?)
4. Do you believe the current wave of consumer activism (largely driven by new techno-media sources) regarding pseudo-food safety issues (e.g., "pink slime" and genetically engineered crops) will play a role in future research funding?
5. What are the 1–3 largest areas that you believe are being overlooked in food safety research grant RFPs, and how would more research in these areas improve public health?
6. What types of research do you deem the most frivolous use of public and industry funds? (i.e., what areas of research will have minimal or no impact on food safety?)
7. What can food industry personnel, academics, government officials, researchers, and anyone with an interest in food safety research do to change "the system?"

Symposium Series on Food Microbiology

Sponsored by the

ILSI North America
Technical Committee on Food Microbiology

in conjunction with the

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

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

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

RT9 Debate - Current Perspectives 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 symposium. The session will cover three topics: "Risk of illness from raw sprout consumption cannot be adequately controlled"; "Effective HACCP programs should negate the need for routine finished product testing yet customer requests for such testing are increasing"; and "Recalls are not an effective tool in the food safety tool box." Each topic will include a 9-min presentation in support of (PRO) followed by a 9-min presentation in opposition to (CON) the proposed question. Each speaker will prepare a minimum of 2–3 slides. Each speaker will have 3-min for extemporaneous rebuttals. A 6-min question/answer session will then follow to allow for audience participation. We will have electronic voter buttons for the audience to use to vote Yes/No/Undecided. A vote is taken at the beginning and again at the end to see whether people's views have changed. 1) Risk of illness from raw sprout consumption cannot be adequately controlled. Pro: Michelle Smith, FDA/CFSAN; Con; Robert Tauxe, CDC 2) Effective HACCP programs should negate the need for routine finished product testing yet customer requests for such testing are increasing. Pro: Tim Freier, Cargill, Incorporated; Con: Tim Jackson, Nestlé USA, Inc. 3) Recalls are not an effective tool in the food safety tool box. Pro: Robert Brackett, Institute for Food Safety and Health, Illinois Institute of Technology; Con: Barbara Kowalcyk, Center for Foodborne Illness Research & Prevention.

Current Perspectives in Food Safety: Recalls are not an Effective Tool in the Food Safety

Pro: ROBERT E. BRACKETT, Institute for Food Safety and Health, Bedford Park, IL, USA

Recalls have for many years been used to remove potentially contaminated products out of commercial distribution, as well as out of people's homes. Although recalls do serve an essential and necessary function, they are fundamentally reactive in nature and an admission that the food safety system for the implicated product has failed. As such, they do little to either prevent or mitigate contamination and are therefore a relatively infective public health tool. Rather, an effective food safety toolbox should rely on those practices and technologies that prevent rather than react to food safety problems.

Con: BARBARA KOWALCYK, Center for Foodborne Illness Research & Prevention, Raleigh, NC, USA

Until recently, federal agencies charged with the oversight of food safety did not have the authority to recall contaminated food products

Risk of Illness from Raw Sprout Consumption cannot be Adequately Controlled

Pro: ROBERT V. TAUXE, Centers for Disease Control and Prevention, Atlanta, GA, USA

While fresh produce is an important part of a healthy diet, consuming produce raw (without a kill step, such as cooking), carries a certain risk. The unique nature and production of sprouts present a special concern with respect to human pathogens compared to other fresh produce. This discussion will explore the risk of illness from raw sprout consumption, history of sprout-associated outbreaks and the adequacy of public and private efforts to control these risks.

Con: MICHELLE A. SMITH, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

While fresh produce is an important part of a healthy diet, consuming produce raw (without a kill step, such as cooking), carries a certain risk. The unique nature and production of sprouts present a special concern with respect to human pathogens compared to other fresh produce. This discussion will explore the risk of illness from raw sprout consumption, history of sprout-associated outbreaks, and the adequacy of public and private efforts to control these risks.

Effective HACCP Programs should Negate the Need for Routine Finished Product Testing Yet Customer Requests for Such Testing are Increasing

Pro: TIMOTHY A. FREIER, Cargill, Minneapolis, MN, USA

The Hazard Analysis and Critical Control Points (HACCP) concept was developed as a logical, systematic approach to food safety largely because the existing system, based mainly on inspection (testing) of finished products, was inadequate to control food safety hazards. For many years, as HACCP was being adopted by food manufacturing facilities, there was a general trend of reduced finished product testing for foodborne pathogens. Testing resources were moved from routine hold-and-release lot testing to more proactive activities such as critical control point validation testing, environmental monitoring and occasional finished product verification testing. Recently, for a variety of reasons, many food manufacturers have increased requirements for end product testing for pathogens by their suppliers. The principles that led to the development of HACCP forty-some years ago remain. Preventing a hazard from occurring continues to be profoundly more effective than trying to find the hazard through testing. Nonhomogeneous distribution and the statistical shortcomings of microbiological sampling and testing have not changed. Increasing synchronization of the supply chain and just-in-time delivery make finished product pathogen testing more difficult and increases the risk for massive recalls if down-stream adulterant testing occurs after the release and distribution of ingredients. We need to swing the pendulum back to the science-based principles of HACCP, placing more reliance on preventive verification activities and less on ingredient pathogen testing.

Con: TIM JACKSON, Nestle USA, Inc., Glendale, CA, USA

S1 The Rise of the Genomes- How Whole Genome Sequencing Will Transform Food Safety

Whole genome sequencing is an emerging technology that is increasingly being used by academia, clinical, public health and food regulatory agency laboratories. It is now possible to sequence the genome of a bacterial strain in approximately one day at a cost of about $100 and the speed is increasing as the cost is going down. The technology is attractive because it may be used to identify, characterize and subtype microbes with precision like never before. It is beginning to enter food laboratories, too. However, it is an emerging technology: sequencing platforms are constantly evolving as is the software that is used to analyze the data and interpretation of the data is rarely straight forward. In this symposium, the basics of the technology will be explained in layman terms, as will the analytical tools and how the technology could be beneficial to the food industry. Implementing a technology as complex as whole genome sequencing is a monumental task and if we want to be able to compare data between laboratories globally, a number of technical, logistical, political and ethical questions needs to be addressed and agreed upon internationally; these aspects will be dealt with by the so-called Global Microbial Identifier initiative. Finally, the public health and food regulatory experience with the technology will be presented as will how it is anticipated the technology will be used in food regulation.

Whole Genome Sequencing – The Basics

KENDRA NIGHTINGALE, Texas Tech University, Lubbock, TX, USA

Advances in genome sequencing technology already allow for a bacterial strain to be sequenced for < $100. Applying recently advanced sequencing technology to food safety and food microbiology will dramatically change how we detect, subtype and characterize foodborne pathogens, spoilage organisms, starter cultures and probiotics. Application of next generation sequencing has and will continue to result in improved detection and subtyping methods, provide new insights into the biology of food-associated microorganisms (i.e., pathogens, spoilage organisms and beneficial bacteria) and facilitate population-based studies on food associated and intestinal microbes. It is clear that next generation sequencing approaches will have a considerable impact on many if not all areas in food microbiology and food safety. The current challenges with these methods do not typically involve the actual generation of genome, transcriptome or metagenomic data, but rather lie in the ability to correctly handle, analyze and interpret the extremely large, terabyte-scale, datasets that are generated by these methods. The ability to translate potentially exciting discoveries facilitated by next generation sequencing to practical outcomes that improve food safety, quality and sustainability, thus requires next generation food scientists and food microbiologists that not only understand food systems and food microbiology, but are also well versed in bioinformatics and other quantitative disciplines. This presentation will cover the history of genome sequencing, the basics of advances in genome sequencing and bioinformatics as well as applications of next generation sequencing to food microbiology.

Bioinformatic Analysis of Whole Genome Sequencing Data and Its Application in the Food Industry

BRUNO SOBRAL, Nestle, Lausanne, Switzerland

A foodborne outbreak of *Escherichia coli*, causing a severe illness called hemolytic-uremic syndrome (HUS) began in Germany in May, 2011. More than 20 people were killed and more than 2,000 were sick. Two isolates from this outbreak were sequenced in real-time with sequencing reads made available. This provided a glimpse of the use case methodologies of the genomic era of epidemiological analysis and further motivated developments in the informatics arena to provide real-time support for such analyses. One responding publicly funded resource, the PAThosystems Resource Integration center (PATRIC, *www.patricbrc.org*), funded by the NIH/NIAID, has further developed, refined and deployed data and metadata structures in support of genomic epidemiology that can be applied to foodborne and other outbreaks. In this talk, I will show cyberinfrastructure available for supporting real-time upload and analyses of data/ metadata related to genomic epidemiology such as in foodborne outbreaks.

The Global Microbial Identifier Initiative

JORGEN SCHLUNDT, The Danish Food Institute, Soborg, Denmark

While previously DNA-sequence based techniques relied on the recognition of short pieces of DNA sequence, the NGS (New Generation Sequencing) technologies now puts within reach for ordinary microbiological labs, the sequencing of enormous amounts of DNA code of microorganisms. The NGS technology enables a generic platform for Whole Genome Sequencing (WGS) of all types of microorganisms, i.e., it represents one uniform testing methodology for all types of microorgansisms within all relevant sectors: Animal, Food, Human Health, etc.

Interestingly, while future use of WGS is likely to boom in developed countries, an even more dramatic change in developing countries creates a potential for significant diagnostic leap-frog in these countries. NGS is a simple one-size-fits-all tool for diagnosis of all infectious diseases, holding the potential to dramatically improve public and animal health and food safety in developing countries.

The Global Microbial Identifier (GMI) initiative is an informal global, visionary task force of scientists and other stakeholders who share the aim of making novel genomic technologies and informatics tools available for improved global diagnostics, surveillance and research. The GMI suggests a global system to aggregate, share, mine and translate genomic data for microorganisms in real-time. This system could include a reference database which would be accessed both for single clinical tasks (simple microbiological identification) as well as for national and international public health surveillance and outbreak investigation and response. The GMI initiative is constructed around an agreed Charter, with a Steering Committee, which also includes representatives from WHO, FAO and OIE. (See Charter and other information at: www.globalmicrobialidentifier.org)

Public Health Experience and Initiatives with Whole Genome Sequencing

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

Whole genome sequencing (WGS) is a technology that likely will transform public health microbiology in a few years. It is a technology that may be used to subtype pathogen with unheard precision, and most phenotypic traits may be predicted from the sequence of their encoding genes. Therefore, WGS has the potential of replacing most of the numerous workflows used in public health laboratories to characterize foodborne pathogens into one consolidated workflow.

The technology has been used to enrich public health investigations of outbreaks of food and waterborne infections in the United States since 2011. In order to increase its utility in such investigations and to understand the genomic diversity of different foodborne pathogens, isolates not related to current outbreak investigations have been sequenced in parallel. A project involving public health, the food regulatory agencies and the National Center for Biotechnology Information to perform national surveillance of listeriosis through real-time WGS of clinical and food isolates was initiated in 2013. It has proven the utility of the technology for outbreak detection, investigation and control. The project will be continued as part of CDC's Advanced Molecular Detection (AMD) initiative, which was built to implement genomics and bioinformatics as a key component in public health activities. As part of AMD, WGS will be implemented in a tiered fashion in PulseNet, the national molecular subtyping network for foodborne disease surveillance in the coming years and used for identification, characterization and subtyping, ultimately replacing virtually all currently used methods.

Challenges and Opportunities for Whole-Genome Sequencing in Regulatory Science: A New Way Forward in the Forensics of Food Safety INVESTIGATION

ERIC W. BROWN, Errol Strain, Peter S. Evans, Ruth E. Timme, Christine E. Keys, Darcy E. Hanes, Palmer A. Orlandi, David G. White, Marc W. Allard, Steven M. Musser, U.S. Food and Drug Administration, College Park, MD, USA

High-resolution forensic tools are essential for aiding in tracing foodborne contamination events back to their source, and in this regard, whole genome sequencing (WGS) is rapidly transforming microbiological subtyping in the food safety laboratory. When coupled with powerful bioinformatic pipelines, accurate and stable genetic changes can be identified across pathogen genomes that can distinguish strains to the source level, including individual farms or facilities and specific geographic locales. This is true even among highly homogeneous strain populations such as *Salmonella* Enteritidis and other salmonellae that have remained largely recalcitrant to differentiation by conventional typing approaches. Numerous published examples illustrate the ability of WGS to discern genetic relatedness of otherwise indistinguishable isolates, and point to WGS as an important tool in the traceability of contamination events. To this end, FDA, in 2012, created an integrated pilot network of state and federal laboratories to use whole genome sequencing to enhance traceback of foodborne pathogens. Known as GenomeTrakr, the network is creating a publically available, global database containing the genetic makeup of thousands of foodborne disease-causing bacteria, including *Salmonella*. At present, WGS impacts regulatory science in FDA's Foods Program in several ways, including: (i) support of traceability efforts during foodborne contamination events; (ii) enhanced regulatory casework for high-risk commodities and compliance standards; and (iii) quality assurance of food microbiological sampling programs. Taken together, early applications of WGS deployments underscore its extraordinary utility in food safety, as well as the potential for complete characterization of bacterial pathogens as they emerge in the food supply.

S11 Parasites in the Food Supply Chain: Emergence or Re-Emergence?

Foodborne Parasites: Transmission, Ecology, Host-pathogen Interaction and Survival in Foods

Foodborne parasites have not received the same level of attention as other bacterial foodborne pathogens. However, recent recalls/outbreaks due to foodborne parasites has raised the need to re-evaluate the prevalence, control, transmission and globalization of the food trade on the role of parasites in the food supply chain. This symposium will provide an overview of the types of foodborne parasites, the mode of transmission, ecology, host relationship prevalence, transmission mitigation strategies and survival in food and water. In addition, this symposium will review a case study on the recent *Cyclospora* outbreak.

YNES R. ORTEGA, University of Georgia, Griffin, GA, USA

Foodborne parasites have been affecting humans since antiquity. There are more than 100 parasites that can cause a range of illnesses in man. The protozoan parasites have been the most responsible for foodborne outbreaks in the U.S. Coccidian parasites infect cells of the susceptible host, and this process is required for the survival of the parasite. Transmission usually occurs through ingestion of contaminated food or water and raw or undercooked food. Several large outbreaks of cyclosporiasis occurred in the late 1990s and were associated with the consumption of fresh imported produce, such as raspberries, basil, lettuce and snow peas. In 2013, imported lettuce and cilantro were implicated in more than 600 cases of cyclosporiasis. Coccidian parasites can infect farm and domestic animals, and the severity of the infection is dependent on the host immune response and prior exposures to the pathogen. Most parasites are highly resistant to environmental conditions, as well as common disinfectant and sanitizers. This poses a challenge to consumers and the food industry in assuring the safety of foods, particularly fresh produce and fruits.

Technologies for the Prevention, Detection and Inactivation of Parasites in Foods

PALMER ORLANDI, U.S. Food and Drug Administration-CFSAN, Silver Spring, MD, USA

Parasites (the helminthes and the protozoa) present difficult challenges to ensuring food safety standards and preventing foodborne illness. Many of these challenges stem from an incomplete understanding of the complex environmental relationship between food matrix and the pathogen; from the types of foods associated with illness (fresh seafood and fresh produce); from the cultural shift toward the consumption of fresh (raw and undercooked) foods that bypass important preparatory practices designed to mitigate the risk of infection for the consumer, and from inadequate surveillance capabilities. Whereas foodborne illness caused by helminthes (typically associated with seafood) are largely sporadic and will only be addressed briefly, illnesses attributed to gastrointestinal protozoa (e.g., *Cyclospora* and *Cryptosporidium*) linked to the consumption of fresh produce, have since the mid-1990s emerged as a growing public health threat and economic concern, and will be the main thrust of this presentation. For these protozoa in particular, environmental factors, e.g., water sources, agricultural practices and personal hygiene play a combinatorial role in transmission and outbreaks of illness. However, the development of effective mitigation strategies are hampered by severe limitations in laboratory capabilities, and the lack of analytical applications necessary to describe the biology of these parasitic pathogens and to support rational food and environmental testing programs. In such a vacuum, mitigation has been based principally on sensible preventive controls from farm-to-table adapted from those developed following the initial outbreaks of cyclosporiasis link to raspberries.

Foodborne Parasitic Disease Surveillance and Control in the United States: Success, Challenges and Opportunities

REBECCA HALL, Centers for Disease Control and Prevention, Atlanta, GA, USA

The Centers for Disease Control and Prevention (CDC) conducts national surveillance for the foodborne parasitic diseases cyclosporiasis and trichinellosis. In addition, *Toxoplasma gondii* seroprevalence data from the National Health and Nutrition Examination Survey (NHANES) are used to estimate the prevalence of infection in the United States. Successes in foodborne parasitic disease control include near-elimination of *Trichinella spiralis* from the domestic pork supply and subsequent rapid decline in human trichinellosis cases. Most reported trichinellosis cases are now associated with consumption of wild game meat. Also, recent *T. gondii* seroprevalence data indicate a continuing downward trend in *T. gondii* infection. However, *Cyclospora cayetanensis* has posed many challenges for food safety and public health response. At least two foodborne cyclosporiasis outbreaks occurred during summer 2013, with 631 laboratory-confirmed cases in 25 states during the outbreak period, but more than two thirds of the cases could not be linked via food exposure histories to either of the two epidemiologically implicated food vehicles, underscoring the need for molecular tools for *Cyclospora* to aid in outbreak detection and response. This presentation will discuss successes, challenges, and opportunities related to the detection, response, and control of parasitic foodborne illnesses and outbreaks.

S47 Threshold of Toxicological Concern (TTC)- A Pragmatic Approval to Determine the Risk of a Chemical Substance of Unknown Toxicity

Continued advances in analytical chemistry techniques lead to greater discovery and detection of very low levels of chemical substances in food. The spectrum and sheer magnitude of low level chemical constituents in the diet presents a challenge to assessing safety. The Threshold of Toxicological Concern (TTC) is a principle that refers to the establishment of a generic human exposure threshold value for groups of chemicals below which there would be a low probability of risk to human health. The concept proposes that such a value can be identified for many chemicals, including those of unknown toxicity based upon their chemical structures. The TTC approach is beneficial to consumers, industry, and regulators by prioritizing necessary testing and extensive safety evaluations to instances when exposure is above a relevant threshold value. This session will review the TTC tool by providing the scientific basis and history of TTC, its applicability to chemical substances found in food and use of TTC by regulatory authorities.

Introduction to Threshold of Toxicological Concern

MITCH CHEESEMAN, Steptoe & Johnson LLP, Washington, D.C., USA

The history of the development of the TTC will be presented, beginning with the earliest attempts to safe human exposure levels to chemical applications with low perceived risks. The evolution of the scientific analysis underpinning the TTC, as well as the evolution of its application to regulatory decision making will be discussed. Finally, TTC will be discussed in terms of current regulatory issues and trends.

Application and Continued Evolution of the TTC and Decision Tree Approaches in the Flavor Industry

SEAN V. TAYLOR, International Organization of the Flavor Industry, Washington, D.C., USA

The Threshold of Toxicological Concern (TTC) has been utilized for the safety evaluation of flavoring substances for more than 30 years. This tool, working in concert with the Cramer/Ford/Hall Decision Tree, has provided a framework for identifying potential concern at an early stage in a safety evaluation. This has resulted in significant efficiency by focusing safety testing resources on substances with highest anticipated toxicity. As our understanding of metabolism and toxicity continues to evolve, it is critical that we update these tools to incorporate new knowledge. An updated Cramer/Ford/Hall Decision Tree is under development by the flavor industry. This project incorporates new information about metabolism and toxicity and attempts to reduce reliance on questions requiring empirical knowledge. Here, we present our progress in this project, the foreseen next steps in relating the updated Decision Tree to the TTC concept, and the potential implications for future flavor safety evaluation.

Determining the Applicability of TTC Approaches to Chemical Substances Found in Foods

RICHARD CANADY, ILSI Research Foundation, Washington, D.C., USA

Threshold of Toxicological Concern (TTC) decision-support methods present a pragmatic approach to using data from well characterized chemicals and protective estimates of exposure in a stepwise fashion to inform decisions regarding low-level exposures to chemicals for which few data exist. It is based on structural and functional categorizations of chemicals derived from decades of animal testing with a wide variety of chemicals. Expertise is required to use the TTC methods, and there are situations in which its use is clearly inappropriate or not currently supported. To facilitate proper use of the TTC, this paper describes issues to be considered by risk managers when faced with the situation of an unexpected substance in food. Case studies are provided to illustrate the implementation of these considerations, demonstrating the steps taken in deciding whether it would be appropriate to apply the TTC approach in each case. By appropriately applying the methods, employing the appropriate scientific expertise, and combining use with the conservative assumptions embedded within the derivation of the thresholds, the TTC can realize its potential to protect public health and to contribute to efficient use of resources in food safety risk management.

Regulatory Overview: What's Next for TTC?

PENELOPE RICE, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

The Office of Food Additive Safety (OFAS) in the U.S. FDA's Center for Food Safety and Applied Nutrition (CFSAN) uses a framework similar to the Threshold of Toxicological Concern (TTC) approach to assess the safety of dietary exposures to migrants from food packaging materials, where the calculated exposures are often extremely low and little or no toxicity data are available. Moreover, structural analysis, such as the Cramer Classification Scheme, upon which the TTC approach is based, has historically been a component of OFAS's risk assessment of both direct food additives and food packaging materials. The following presentation will discuss how the thought process and scientific principles of the TTC approach are incorporated into OFAS's guidelines for human health risk assessment of food additives and food packaging materials and will summarize OFAS's ongoing scientific projects in the areas of TTC and updating the Cramer Classification Scheme.

Technical Abstracts

T1-01 Development of a Single Assay Comprising Nano-immunomagnetic Separation of *Escherichia coli* O157:H7 from Apple Juice Followed by Surface Enhanced Raman Scattering-based Detection

PRATIK BANERJEE, Roya Najafi, Shubhasish Mukherjee, Anup Sharma
The University of Memphis, Memphis, TN, USA

Introduction: The successful detection of foodborne pathogens is dependent on efficient separation and concentration of target organism from the food matrices. Immunomagnetic separation (IMS) is one of the commonly used methods for isolation of pathogens from food and beverages. However, traditional IMS is time consuming and requires additional steps to confirm the identity of the captured microbes. Therefore, the need to develop a rapid IMS procedure enabling both microbial separation and identification in a single assay is highly desirable.

Purpose: The purpose of this study was to develop a method combining gold-coated iron nanoparticle-based IMS and surface enhanced Raman spectroscopy (SERS)-based detection in a single assay for screening of *Escherichia coli* O157:H7 from model liquid food.

Methods: Magnetite-gold (Fe_3O_4/Au) nanoparticles (MNP) were synthesized using Polyethyleneimine (PEI). The capture antibody (cAb) was immobilized on gold coated MNP. These cAb-MNP were used in the separation and concentration of the *E coli* O157:H7 cells from spiked apple juice samples. The capture efficiency was obtained in different *E. coli* O157:H7 concentrations (10^2–10^8 CFU /ml). The specificity of the assay was tested by using a group of different gram positive and gram negative bacteria. For detection of target pathogen, SERS labels were prepared by conjugating gold nanoparticles with Raman reporter molecules and the detector antibody (dAb). Two different Raman reporter molecules were examined. Gold-Raman label-dAb was interacted with gold coated MNP-cAb-*E. coli* O157:H7 complex. Following IMS and washing, the nano-aggregates were directly analyzed using a Raman spectrometer.

Results: The percentage of captured bacteria (expressed as capture efficiency, CE) for *E. coli* O157:H7 was found to be about 85-94%. No cross reactivity was observed with background non-target organisms at a concentration of 10^6 CFU /ml. There was a significant difference in the mean CE of bacteria captured by MNP and commercially sourced immunomagnetic microbeads ($P < 0.05$). The limit of detection of bacterial cell in apple juice using nanoparticles was 10^2 CFU/ml.

Significance: This newly developed IMS method, employing Fe_3O_4/Au nanoclusters, presents a convenient way for isolation as well as detection of target pathogen (*E. coli* O157:H7) in food sample. The method is rapid and sensitive to target organisms with a total assay time of less than 60 minutes.

T1-02 TaqMan-based Multiplex Real-time PCR Assays for the Detection and Quantification of the Six Major Non-O157 Shiga Toxin-producing *Escherichia coli* in Cattle Feces

PRAGATHI BELAGOLA SHRIDHAR, Lance Noll, Baoyan An, Xiaorong Shi, TG Nagaraja, Jianfa Bai
Kansas State University, Manhattan, KS, USA

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are responsible for severe foodborne illnesses in humans. Non-O157 STEC, particularly O26, O103, O111, O45, O121 and O145, are gaining attention in recent years as they are responsible for more than 70% of human STEC infections. Cattle harbor non-O157 STEC in the gut and shed in the feces. Molecular detection methods, particularly real-time PCR, for non-O157 *E. coli* detection in cattle feces have not yet been developed and validated.

Purpose: To develop multiplex real-time PCR assays to detect and quantify the six major non-O157 STEC, O26, O103, O111, O45, O121 and O145, in cattle feces.

Methods: Two sets of serogroup-specific assays targeting O antigen genes, O26, O103, O111 (assay 1) and O45, O121, O145 (assay 2) were developed. Specificity of the assays was assessed by testing a panel of positive and negative *E. coli* control strains. Sensitivity of the assays was determined with 10-fold serial dilutions of pure cultures and culture-spiked fecal samples. Spiked fecal samples were enriched in *E. coli* broth for 6 h at 40°C. Fecal DNA extracted before and after the enrichment were used as templates for real-time PCR. Assays were compared to conventional PCR and culture-based method of detection by analyzing 225 cattle fecal samples.

Results: The assays were specific for the target genes and no cross-reactions were observed. The detection limits of the assays were 10^3 CFU/ml, 10^4 CFU/g and 10^2 CFU/g for pooled pure cultures, before and after enrichment of fecal samples spiked with the six non-O157 STEC, respectively. Real-time PCR assays (171/225) detected a higher number of positive samples than conventional PCR (55/225) and culture-based (110/225) methods.

Significance: The multiplex real time PCR assays developed are sensitive diagnostic tools for the detection and quantification of the six non-O157 *E. coli* serogroups in cattle feces.

T1-03 Label-free Optical Biosensor to Monitor Antibiotic Resistance in Bacterial Pathogens

ATUL SINGH, Arun Bhunia
Purdue University, West Lafayette, IN, USA

Introduction: Antibiotics are used for treatment of microbial diseases in humans and animals, and as growth promoting agents during animal meat production. Extensive and indiscriminate use of antibiotics resulted in the emergence of antibiotic resistance in microbes, and contributes to a large number of nosocomial or community acquired infections and mortalities. Current molecular and immunological assays to screen antibiotic resistance in pathogens are arduous, need technical expertise and resource to perform the analysis. We used light scattering device designated BARDOT (**ba**cterial **r**apid **d**etection using **o**ptical scattering **t**echnology) as a rapid on-plate antibiotics resistance screening tool.

Purpose: To test BARDOT as a screening tool for antibiotic resistance in bacterial pathogens.

Methods: BARDOT uses a red diode laser (635 nm, 1 mW) to illuminate bacterial colonies of 1.2 ± 0.1 mm diameter to generate the signature scatter patterns. Cultures of *Salmonella*, *Staphylococcus*, *Enterococcus*, *Klebsiella* and *Acinetobacter* (total strains, n = 15) were plated with and without antibiotics on nutrient agar plate (BHI, TSA, LB, and NB) for inclusive growth of all pathogens. Scatter patterns of colonies were acquired and images were analyzed using quantitative image classifier. The results were represented as positive predictive value (PPV). Quantitative reverse transcriptase PCR (qRT-PCR) was performed to confirm the expression of antibiotic resistance gene (*aadA*, *mecA*) in a dose dependent manner.

Results: BARDOT generated differentiating scatter pattern for bacterial colony grown on antibiotic containing nutrient agar plate compared to agar media without antibiotics in a dose dependent manner. Scatter pattern profile of colonies on plate containing antibiotic or without antibiotic

were differentiated with >90% PPV. qRT-PCR results verified increased expression of antibiotic resistance and corroborated with the observed scatter patterns for the antibiotic treatment in a dose dependent manner.

Significance: BARDOT could be used as a real-time, label-free on-plate colony screening tool for antibiotic resistance in bacterial pathogens.

T1-04 Withdrawn

T1-05 Withdrawn

T1-06 Evaluation of Universal Pre-enrichment Broth with Novel *Listeria* and *Salmonella* Rapid Methods for Dual Pathogen Detection from Environmental Sponges

CHRIS LOPEZ, Bharath Bramhmanda, Ron Johnson, J. Stan Bailey, Ray Turnley
Food Safety Net Services, San Antonio, TX, USA

Introduction: Conventional methods for detecting *Salmonella* and *Listeria* spp. traditionally require separate enrichments for each pathogen. Universal Pre-enrichment Broth (UPB) in combination with VIDAS® UP (SPT and LPT) phage based rapid methods was evaluated as a dual enrichment medium for detecting *Salmonella* and *Listeria*.

Purpose: To validate novel alternative methods for detecting both *Salmonella* spp. and *Listeria* spp. in environmental samples from one enriched sponge using UPB.

Methods: Stainless steel surfaces were spiked with a cocktail of *Listeria monocytogenes* and *Salmonella* Typhimurium, from which one set of sponges were enriched in UPB and compared to sponges that were enriched with BPW for *Salmonella* spp. and sponges enriched with UVM for *Listeria* spp. UPB enrichments were analyzed by the phage based next day detection methods and 48h immunoassay detection methods. The UPB methods were compared to AOAC-approved BPW and UVM 48h enrichment methods.

Results: For *Listeria* detection; from the UPB enrichment, the phage-based assay detected16 positives out of 30 spiked samples, the 48 h immunoassay detected 15 of 30 spiked samples compared to 12 out of 30 spikes samples for the AOAC-UVM/VIDAS approved 48h method. For *Salmonella* detection; from the UPB enrichment , the phage based assay detected14 positives out of 30 spiked samples, the 48h immunoassay detected 15 of 30 spiked samples compared to 16 out of 30 spikes samples for the AOAC-BPW/VIDAS approved 48 h method. The UPB enriched detection methods were statistically equivalent to both AOAC-approved single enrichment methods.

Significance: The VIDAS® UP *Salmonella* (SPT) and VIDAS® UP *Listeria* (LPT) next day detection methods from a single UPB enriched sponge were equivalent to the 48h enrichment methods for detecting *Salmonella* spp. and *Listeria* spp. from stainless steel surfaces.

T1-07 Advancing Metagenomics Analysis of Viruses in Irrigation Water and Field-grown Lettuce

TIONG GIM AW, Samantha Wengert, Joan Rose
Michigan State University, East Lansing, MI, USA

Introduction: An increasing number of foodborne outbreaks associated with fresh produce has been observed and this indicates the vulnerability of our food systems to contamination and public health risk. The host specificity of viruses suggests that they could be promising library-independent tool to determine the major microbial sources in food system. The rapid development of culture- and sequence-independent metagenomics technologies presents an opportunity for generating an improved understanding of the viral communities associated with fresh produce and irrigation water.

Purpose: The objective of this study was to characterize viral communities and assess their diversity in irrigation water and lettuce from the field using metagenomics with high throughput sequencing technology (Illumina sequencing).

Methods: Viruses were concentrated from 100l of irrigation water (n = 6) using hollow fiber ultrafiltration method. For romaine and iceberg lettuce (n = 42), viruses were recovered using Tris-glycine buffer (pH 9.5) followed by precipitation with polyethylene glycol. Viral nucleic acid was extracted and pooled for Illumina sequencing (HiSeq 2500). Bioinformatics approaches were used to analyze the viral metagenomics fingerprints.

Results: Average recovery of bacteriophage P22 from romaine and head lettuce in seeded experiments ranged between 42 and 73%. The proof-of-concept metagenomics study using wastewater showed that 30% of the viruses in sewage were novel. The virome of sewage was dominated by bacteriophages (48%) and human viral pathogens comprised 1% of total known virus sequences. Metagenomics analysis of the viromes of irrigation water and various lettuce samples will be summarized and contrasted to sewage environments.

Significance: The new science of viral metagenomics could be used to discover potential novel viral indicators or viral genetic markers to correlate with the supply of safe and healthy food. This represents a significant advancement in how we evaluate the quality and safety of fresh produce.

T1-08 A New *In Situ* Capture-qRT-PCR (ISC-qRT-PCR) Method for an Alternative Approach to Determine Inactivation of Human Norovirus

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Introduction: Human noroviruses (HuNoVs) are the major cause of epidemic non-bacterial gastroenteritis. Although quantitative real-time RT-PCR (qRT-PCR) is widely used for detecting HuNoVs, it only detects the presence of viral RNA and does not indicate viral infectivity. Human blood group antigens (HBGAs) have been identified as receptors/co-receptors for both HuNoVs and Tulane Virus (TV), and are crucial for viral infection. We propose that viral infectivity can be evaluated with a molecular assay based on receptor-captured viruses.

Purpose: We employed TV as a HuNoV surrogate to validate the HBGA-based ISC-qRT-PCR method against the $TCID_{50}$ method, and applied the validated ISC-qRT-PCR method to determine the inactivation parameters for HuNoV.

Methods: We bound HBGAs to immuno-PCR well-strips (Nunc Top-Yield), and used the immobilized receptors to concentrate TV or HuNoV. This is followed by amplification of the captured viral genomic signal (CVGS) by *in situ* qRT-PCR.

Results: We first demonstrate that this ISC-qRT-PCR method could effectively concentrate and detect TV. We then treated TV under either partial or full-inactivation conditions, and measured the remaining CVGS by ISC-qRT-PCR and tissue-culture-based amplification method ($TCID_{50}$). We found that the ISC-qRT-PCR method could be used to evaluate TV inactivation caused by damage to the capsid, and study interactions between the capsid and viral receptor. Heat, chlorine, and ethanol treatment primarily affect the capsid structure, which in turns affects the ability of the capsid to bind to viral receptors. Inactivation of TV by these methods could be reflected by ISC-qRT-PCR method and confirmed by $TCID_{50}$ assay. However, the loss of infectivity caused by damage to the viral genome (such as from UV irradiation) could not be effectively reflected by this method. With this validated

method, we further determined inactivation parameters for HuNoV. HuNoV could be inactivated by heat at 72°C for 4 minutes, by chlorine at a final concentration of 16 ppm in less than 1 minute, and by UV irradiation at 1 J/cm^2. However, ethanol had a limited effect on high-titer samples of HuNoV (> 10^{3}copies/sample).

Significance: We demonstrated that ISC-qRT-PCR provides an alternative approach to determine the inactivation of HuNoV. A particular advantage of the ISC-qRT-PCR method is that it is a faster and easier method to effectively recover and detect viruses, as there is no need to extract viral RNA, nor transfer the captured virus from magnetic beads to PCR tubes for further amplification. Therefore, ISC-qRT-PCR can be easily adapted for use in automated systems for multiple samples.

T1-09 Detection and Identification of Anabolic Steroids in Over-the-counter Nutritional Supplements

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Introduction: During 2013, the New York State Department of Health (NYSDOH) investigated an outbreak among 29 persons reporting adverse health effects, including hirsutism, fatigue, myalgias, anxiety, and elevated liver and cholesterol tests. Of patients affected, one was hospitalized for acute liver injury and one woman exhibited signs of masculinization. NYSDOH received reports of other hundreds of potentially affected persons. The epidemiologic investigation implicated consumption of dietary supplements including vitamin B-50 (term used by the manufacturer) and multimineral supplements from the same supplier.

Purpose: This study was to determine organic components and potential contaminants in the dietary supplement samples and to investigate whether these supplements were responsible for the adverse health effects observed.

Methods: Comprehensive chemical analyses of multiple marketed lots of the supplements were performed. Organic components and contaminants in the dietary supplement samples were extracted before analysis by gas chromatography–mass spectrometry, time-of-flight mass spectrometry, and liquid chromatography coupled to electrospray tandem mass spectrometry.

Results: Analyses revealed the presence of significant levels of methasterone (17β-hydroxy-2a,17a-dimethyl-5a-androstane-3-one), an anabolic steroid and Schedule III controlled substance, as well as other anabolic steroids (e.g. methylstenbolone).

Significance: Compounds in the dietary supplements were suspected to have caused the liver toxicity and masculinizing effects that were observed in these patients. Without cessation of consumption, certain adverse health effects can be permanent and have the potential of increasing morbidity and mortality.

T1-10 Trace Analysis of Mycotoxins in Food Matrices by Solid Phase Extraction - Liquid Chromatography Tandem Mass Spectrometry

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Introduction: In February 2013, several Western European countries, including Romania, Serbia, Croatia and Hungary reported nation-wide contamination of milk for human consumption with aflatoxins. Aflatoxins are a type of mycotoxins, which are well known as toxic secondary metabolites produced by fungi and are often found in agricultural commodities. Many mycotoxins are genotoxic, cytotoxic, and carcinogenic. Trichothecenes, because of their high toxicity to humans, may be used as bioweapons to produce lethal casualties in terrorist acts.

Purpose: The purpose of this study was to develop a method for analysis of mycotoxins including aflatoxins B1, B2, G1, G2, ochratoxin A, and T-2 toxin in liquid milk and general cereal grains such as corn, wheat, and oats.

Methods: The mycotoxins were extracted in acetonitrile-water containing 1% (v/v) formic acid followed by a clean-up procedure using C18 solid phase extraction (SPE) cartridges prior to the analysis by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI/MS/MS).

Results: Selected matrices fortified at 10 and 100 ng/g provided mean recoveries ranging from 76% to 124% with average method detection limits ranged from 1 to 4 ng/g matrix.

Significance: Results from this study will not only provide a valuable resource for the identification and quantification of mycotoxins in food products but also be important in responding to emergencies involving their widespread contamination.

T1-11 Sterilization Enhancement of Low-concentrated Ozone Gas Using Micro-bubbles by Surface-active Agents

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Introduction: Ozone gas is a strong oxidant. Ozone has possibilities of being used as an alternate technique of chloride reagents that is used to sterilize fresh-cut vegetables, but subtle ozone water does not have a sufficient effect on sterilization. Thus, to enhance sterilization performance of ozone is required.

Purpose: The objective of this study is to develop new technology that enhances sterilization performance of low-concentrated ozone gas using micro-bubbles by surface-active agents.

Methods: The testing bacteria solution was prepared by mixing 20 g of commercial non-bleach bean sprouts and 90 ml of sterilized saline with a homogenizer. This solution included 5 log CFU/ml of viable bacteria, and pH was 6.5. Two types of ozone solutions were evaluated; 1) The mixed solution that consists of dissolved ozone water and the bacteria solution. 2) The micro-bubble ozone solution that was 400 ml of bacteria solution including 5 g/l triacetin as a surface-active agent, and 0.063 mg/l of ozone gas was aerated into solution with a flow rate of 1.0 l/min for 2.5 minutes using a porous glass. Viable bacteria surviving in these solutions were counted to evaluate the sterilization performance.

Results: In the case of mixed solution that initially has dissolved 0.5 mg/l of ozone, 3.83 ± 0.02 log CFU/ml of viable bacteria was detected. Adding triacetin to this solution, the viable bacteria was 3.56 ± 0.04 log CFU/ml, and addition of triacetin showed little effect on performance. On the other hand, in the case of micro-bubble ozone solution, viable bacteria were not detected. Because the amount of supplied ozone of micro-bubble treatment was sufficiently lower than that of mixed solution, it can be assumed that micro-bubble generation by surface-active agents enhance the sterilization performance of ozone gas.

Significance: Micro-bubble generation utilizing surface-active agents is an effective way to enhance sterilization performance of ozone.

T1-12 Microplate Immunocapture (IMC): A New Solution for the Isolation/Concentration of *Escherichia coli* O157:H7 from Food Samples

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Introduction: Immunocapture (IMC) is used for the concentration of pathogenic bacteria from food product to facilitate their detection. Immuno-magnetic separation has been traditionally the reference method for capturing foodborne pathogen bacteria.

Purpose: We have developed a new method for the immunocapture of pathogenic bacteria based on 96-well microplate coated with specific antibodies. The objective of this study was to demonstrate the efficiency of this method in capturing *Escherichia coli* O157 in enriched food samples.

Methods: After adding 100µl of enrichment broth (BPW) into the wells, antibodies are capturing present target bacteria. Then, after a washing step, a subculture is performed (3 to 5 hours) in the wells with addition of fresh enrichment medium (100µl mTSBn) for increasing the number of target bacteria. The efficiency of the system was assessed by performing immunocapture on food samples (ground beef / raw milk cheese, 25-g sample size), artificially contaminated with *E. coli* O157:H7. Naturally contaminated beef samples found positive by PCR screening were also confirmed by IMC plate. Tests were also done with magnetic beads.

Results: For ground beef samples, IMC with microplate allowed the capture of about 5.4 10^5 CFU of *E. coli* O157 when magnetic beads recovered 2.7 10^3 CFU. For both raw milk cheeses, IMC microplate captured about 3.2 10^5 CFU (goat cheese) and 2.6 10^5 CFU (Mont d'Or) of *E. coli* O157 when magnetic beads recovered about 6.2 10^3 CFU (goat cheese) and 2.7.10^4CFU (Mont d'Or). IMC microplate allowed the confirmation of 33 out of 38 positive PCR screening for the naturally contaminated meat samples.

Significance: IMC microplate was shown effective for the capture isolation of *E. coli* O157 bacteria from enriched meat samples. The subculture step done into the wells, after immunocapture, contributes to a good confirmation rate.

Technical

T2-01 Efficacy of Three Light Technologies for Reducing Microbial Populations

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Introduction: In recent years, non-thermal technologies have shown potential as alternative disinfection technologies. Three light technologies (e.g., High-Intensity Narrow-Spectrum (HINS) light 395 ± 5 nm, continuous UV light and High Intensity Light Pulses (HILP)) are non-thermal technologies which can be used to inactivate bacteria, yeasts, molds and even viruses.

Purpose: The purpose of the current study was to evaluate the effectiveness of three non-thermal light technologies (NUV-Vis, Continuous UV and HILP) on their ability to inactivate *Escherichia coli* K12 and *Listeria innocua*.

Methods: Experiments were conducted using *E. coli* K12 (DSM 1607) and *L. innocua* (NCTC 11288). The suspensions containing both *E. coli* and *L. innocua* were assessed for susceptibility to three light technologies in a liquid matrix (MRD). A wide spectrum of dosages, varying between 0.18-106.2 J/cm^2, was used for all the light treatments.

Results: The results of the present study show that the HIPL treatment inactivated both *E. coli* and *L. innocua* more rapidly and effectively than either continuous UV-C or NUV-vis treatments. With HILP at a distance of 2.5 cm from the lamp, *E. coli* and *L. innocua* populations were reduced by 3.07 and 3.77 log CFU/ml, respectively, after a 5 sec treatment time, and were shown to be below the limit of detection (< 0.22 log CFU/ml) following 30 sec exposure to HILP (106.2 J/cm^2).

Significance: These data suggest that short treatment times for decontamination efficiency would be an important factor related to productivity in the food industry. The findings presented here suggest the expansion of the aforementioned light technologies on food decontamination. Thus, these alternative non-thermal disinfection light techniques could find potential application for decontamination in the food industry.

T2-02 Effect of Organic Acids on Inactivation of Selected Foodborne Pathogens Using 461 nm Light Emitting Diodes (LEDs)

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Developing Scientist Competition

Introduction: Inactivation of foodborne pathogens using light emitting diodes (LEDs) has received increased attention in recent times. While LEDs have been shown to produce an antibacterial effect on foodborne pathogens, the effect of the conditions that the pathogens may encounter in the food matrix on such inactivation is yet unknown. With this in mind, the presence of organic acids in the food matrix is an important factor which is likely to affect this inactivation.

Purpose: This study aimed to determine the effect of organic acids on the LED inactivation of foodborne pathogens and in the process, better understand the application and suitability of this novel technology.

Methods: LEDs with a peak wavelength of 461 nm were used to illuminate four foodborne pathogens – *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus*. These pathogens were placed in tryptone soya broth, whose pH was adjusted to 4.5 using citric, malic or lactic acid. Survivor curves were plotted and modelled using the Weibull Model. The D-values were subsequently calculated and compared using ANOVA.

Results: LED illumination significantly ($P < 0.05$) inactivated all four pathogens under the presence of the three acids, showing reductions of 2 – 5 log CFU/ml. Lactic acid was found to be the most effective in aiding the LED inactivation, while malic acid was found to be the least effective. This trend was reflected by the decimal reduction times (D-values) observed for these acids. For example, the D-values observed for *E. coli* O157:H7 were 3.66 ± 1.61, 4.05 ± 0.7 and 0.7 ± 0.14h using citric, malic and lactic acids respectively during the LED illumination, exhibiting significant ($P < 0.05$) difference among the D-values.

Significance: The results of this study suggest that the effectiveness of the LED treatment is likely to be different on foods of similar pH but having different predominant acids.

T2-03 Thermal Inactivation of Hepatitis A Virus in Turkey Deli Meat

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Introduction: Hepatitis A virus (HAV) has been implicated in several foodborne outbreaks linked to the consumption of pre-sliced ready-to-eat deli meats. Therefore, proper inactivation of HAV prior to consumption is essential.

Purpose: The purpose of this study was to (i) characterize the thermal inactivation behaviour of HAV in deli meat, (ii) compare first-order and Weibull models in describing the data in terms of selected statistical parameters, and (iii) calculate z-values and activation energy by each model.

Methods: Turkey deli meat purchased from a local market was cut into circular pieces (diameter = 3 cm). One-hundred µl of HAV with an initial titer of 7.27 ± 1.46 log PFU/ml was used to inoculate deli meat surfaces, and allowed to air-dry for 30 min under the biosafety cabinet. Inoculated samples were vacuum-sealed in a plastic bag to -100 kPa with a MultivacA300/16 vacuum-packaging unit. Sealed bags were placed into a holding unit prior to heating in a thermostatically controlled water-bath with temperature monitored by thermocouples. Survivor curves (D-values) and thermal death curves (z-values) were generated for different treatment times (0-6 min) at different temperatures (50, 56, 60, 65 and 72°C). Weibull and first-order models were compared to describe survivor curve kinetics and thermal death times. A comparison test (ANOVA, Post Hoc test) was used to analyze the effects of the temperature and time on survival ratio.

Results: Calculated D-values from the first-order model (50-72°C) ranged from 1.01 to 42.08 min for HAV. Using the Weibull model, the t_D for HAV to destroy 1 log (D = 1) at the same temperature was 1.0 to 31.10 min. At 72°C, the required treatment time to achieve 6-log reduction was 6.06 min for the first-order model, and 6.92 min for the Weibull model. The z-value for HAV was 12.90 ± 0.64°C for first-order model and 16.36 ± 3.87°C for the Weibull model. The calculated activation energies for the first-order model and the Weibull model were 173 and 151 kJ/mole, respectively.

Significance: This study provides novel and precise information on thermal inactivation of HAV in turkey deli-meat enabling more reliable thermal process calculations to inactivate HAV and control outbreaks.

T2-04 Determination of the Thermal Inactivation Kinetics of *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* in Fettuccine Alfredo

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Introduction: Several foodborne illness outbreaks have been linked to ready-to-eat foods, such as pasta dishes. Mild heat processing can be a reliable method of inactivating pathogens, but it is necessary to determine the thermal inactivation kinetics of pathogens in food systems to design efficient thermal processing methods.

Purpose: The objective of this study was to determine the thermal inactivation kinetics of selected pathogenic bacteria in a fully-cooked pasta with sauce.

Methods: Five-strain cocktails of *Escherichia coli* O157:H7, non-O157:H7 *E. coli*, *Salmonella enterica* and *Listeria monocytogenes* were mixed with a commercial Alfredo sauce. Fettuccine noodle pieces (3.8 cm length) were mixed 1:1 (w/w) with the sauce and allowed to sit for 30 min before vacuum packaging in polyethylene-nylon bags. Bags were placed in a holder and submerged in a circulating water bath at 56, 58 or 60°C. Following heating for appropriate times, samples were placed in an ice-bath, blended with phosphate buffered saline, then serially diluted and spread-plated on tryptic soy agar plates. Survivors from triplicate experiments were enumerated after 24h at 37°C or 48h at 32°C (*Listeria*). D- and z-values were determined using a first-order model.

Results: For *Salmonella*, *Listeria, E. coli* O157 and non-O157, D-values (min) at 56°C were 3.75 ± 0.39, 7.58 ± 1.04, 7.66 ± 0.04 and 7.47 ± 0.40, at 58°C, 1.07 ± 0.06, 2.07 ± 0.06, 3.39 ± 0.14 and 5.03 ± 0.39, and at 60°C were 0.46 ± 0.01, 1.09 ± 0.32, 1.20 ± 0.19 and 1.28 ± 0.22 min, respectively. z-values for *Salmonella*, *Listeria monocytogenes*, *E. coli* O157 and non-O157 were 4.42 ± 0.33, 4.82 ± 0.74, 4.96 ± 0.42 and 5.20 ± 0.39°C, respectively.

Significance: These results show the difference in thermal inactivation kinetics of the selected pathogens in pasta with sauce. These data will be useful to the food industry in developing mild thermal treatments, such as microwave pasteurization, to produce safer consumer products.

T2-05 Growth and Control of Pathogens in Biofilms on the Surface of Stainless Steel by Chemical Sanitizers and Temperature

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Developing Scientist Competition

Introduction: Foodborne pathogens can form biofilms, be enclosed in a matrix of primary polysaccharides and are more resistant to environmental challenges.

Purpose: To investigate the biofilm formation of foodborne pathogens on the surface of stainless steel and to evaluate the efficacy of temperature and chemical sanitizers on the removal of biofilms.

Methods: Mixtures of *L. monocytogenes, S.* Typhimurium, and STEC were separately inoculated on stainless steel coupons. The coupons were then either treated by heat (60, 80, and 100°C) for 10 min or by a chemical sanitizer for 10 min, or by both. The sanitizers used were a quaternary ammonium compound, lactic acid, sodium hypochlorite, hydrogen peroxide, levulinic acid (LVA), sodium dodecyl sulfate (SDS), and three different concentrations of LVA plus SDS. The treated coupons were subsequently enriched in different broths. The attached cells were dislodged by using the bead vortex method.

Results: Ca. 8.6 to 9.2 log CFU/coupon of pathogens grew in the biofilms after 72h of incubation at 100% relative humidity and 21°C. Inactivation was greater when the temperature was increased. Increasing the temperature to 80°C resulted in significant ($P < 0.05$) reduction of 1.0, 1.4, and 1.7-log reduction of the pathogens, respectively. The three pathogenic cell numbers were reduced by 0.3 to > 6.9 log CFU/coupon within 10 min when treated with QAC (150 ppm), SHC (100 ppm), HP (2%), LVA (3%), SDS (2%) and LVA+SDS (0.5% LVA+0.05% SDS, and 1% LVA+0.1% SDS). More ($P < 0.05$) colonies were recovered from TSA than selective plates after LA (3%) treatment. When 80°C+LA (3%) and 3% LVA+2% SDS were applied, all three pathogenic cell numbers were reduced to an undetectable level (negative by enrichment cultures).

Significance: Results revealed that 80°C+ LA (3%) or 3% LVA+ 2% SDS sanitizer can effectively reduce foodborne pathogenic biofilms by >6.9 log CFU/coupon on stainless steel.

T2-06 Effect of Heating Medium on the Thermal Inactivation Kinetics of *Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica*: Buffer vs. a Spinach Model System

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Introduction: There has been an increase in foodborne illness outbreaks caused by pathogens in leafy vegetables. Mild thermal processes, e.g., blanching, may be an effective tool for reduction of pathogens in these products. Evaluating thermal inactivation kinetics *in vitro* and in foods is important for determining adequate processing times and temperatures, as thermal resistance changes in food matrices.

Purpose: This study aimed to compare the thermal inactivation kinetics of *Listeria monocytogenes* and *Salmonella enterica* in buffer and spinach.

Methods: Five-strain cocktails of each microorganism in phosphate buffered saline in 2 ml vials or blended spinach (1:1 (w/v) with water) vacuum-sealed in polyethylene-nylon bags were exposed to 56, 58 and 60°C in a circulating water bath with temperatures monitored by thermocouples. Following heating for appropriate times, samples were removed, placed in an ice bath, then serially diluted and spread-plated on tryptic soy yeast extract agar plates. Colony forming units (CFU) were recorded after 24h at 37°C (*Salmonella*) and 48h at 32°C (*Listeria*). Each experiment was replicated thrice. D- and z-values were calculated using a first-order model.

Results: D-values for *Listeria* in PBS were 4.42 ± 0.16, 1.45 ± 0.22 and 0.58 ± 0.04 min at 56°C, 58°C and 60°C, respectively while for *Salmonella* they were 2.04 ± 0.24, 0.55 ± 0.03, 0.21 ± 0.01 min, respectively. D-values were higher in spinach: 11.77 ± 2.18, 4.48 ± 0.92 and 1.22 ± 0.12 min at 56°C, 58°C and 60°C, respectively, for *Listeria* and 3.51 ± 0.06, 1.19 ± 0.02 and 0.47 ± 0.06 min, respectively, for *Salmonella*. z-values were 4.52°C and 4.06°C in PBS and 4.06°C and 4.59°C in spinach for *Listeria* and *Salmonella*, respectively.

Significance: These results indicate that heating medium affects thermal inactivation kinetics. These data will potentially aid the food industry in developing appropriate mild heating processes, such as blanching or microwave processes, to eliminate foodborne pathogens.

T2-07 Acid Adaptation Enhances *Salmonella* Enteritidis Acid and Heat Resistance Due to the Decreased Membrane Fluidity But Not Upregulation of the Stress Related Genes

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Introduction: *Salmonella* Enteritidis is one of the most common causes of human salmonellosis reported worldwide. Antimicrobials, like lactic acid (LA) and trisodium phosphate (TSP), have been approved to reduce foodborne pathogens in animal carcasses. However, adaptation to the sublethal concentration of these antimicrobials may render pathogens greater resistance towards lethal conditions and become more virulent.

Purpose: This study aimed to investigate whether acid or alkaline adaptation alters *Salmonella* acid/heat resistance and virulence gene expression, as well as, to find out whether the alternation in *Salmonella* resistance was due to the changes in membrane lipid composition/expression of stress-related genes or both of them.

Methods: *S*. Enteritidis was cultivated at different pHs (5.3-9.0) adjusted by LA or TSP. Adapted cells were subject to simulated gastric fluid (pH=2.0) and thermal treatment (54, 56, 58, and 60 °C) to determine its acid/heat resistance. *D*-values were calculated based on survivor curves. Membrane lipid composition was determined by gas chromatography. The transcription levels of stress-related genes (*rpoS* and *rpoH*) and virulence-related genes (*spvR*, *hilA*, *sefA*, and *avrA*) were evaluated by real-time PCR. Mean values were compared using ANOVA.

Results: Results showed that cells adapted at pH 5.3 had significantly ($P < 0.05$) higher acid (*D*-value = 7.9 min) and heat resistance ($D_{60\,^{\circ}C}$-values = 0.87 min), followed by the control and alkali-adapted cells. The increased resistance was correlated with the decreased membrane fluidity; whereas no upregulation of *rpoS* and *rpoH* was found in acid-adapted cells. About 6.0, 2.1, and 2.5-fold upregulation of *spvR*, *avrA*, and *hilA* were observed in cells adapted to pH 9.0, whereas *sefA* had its highest expression level in the control cells.

Significance: This study indicates that the regulation of the cytoplasmic membrane rather than the stress-related genes may play a more crucial role in conferring acid and heat resistance on acid adapted *S*. Enteritidis.

T2-08 Effect of NaCl on Enterotoxin Production and Invasion Efficiency of *Staphylococcus aureus*

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Introduction: Although many processed foods contain NaCl and the foods are occasionally contaminated with *Staphylococcus aureus*, which results in foodborne illness, the effect of NaCl on the pathogenicity of *Staphylococcus aureus* has not been studied.

Purpose: The objective of this study was to evaluate the effect of NaCl on the enterotoxin production and Caco-2 cell invasion efficiency of *S. aureus*.

Methods: *Staphylococcus aureus* strains NCCP10826, ATCC27664, ATCC13565, ATCC23235, and ATCC14458 were inoculated in to 10 ml tryptic soy broth plus 0, 2, 4, and 6% NaCl at 35°C for 24h, followed by plating 0.1ml of the culture on tryptic soy agar plus 0, 2, 4, and 6% NaCl. After incubation at 35°C for 24h, the colonies of *S. aureus* on plates were suspended in phosphate buffered saline and diluted to OD_{600}=0.1. The diluents were further used to measure a staphylococcal enterotoxin by a immunoassay and to evaluate Caco-2 cell invasion efficiency.

Results: For enterotoxin production, there were no correlations between NaCl concentration and enterotoxin production for all *S. aureus* strains. Of five strains, the adhesion efficiency of *S. aureus* ATCC14458 was significantly increased ($P < 0.05$) up to 6.44% at 2% NaCl. In addition, the invasion efficiency of *S. aureus* ATCC14458 significantly increased ($P < 0.05$) up to 9.1% at 6% NaCl, which was 2.3 times higher ($P < 0.05$) than that of 0% NaCl.

Significance: These results suggest that NaCl in food may increase the cell invasion efficiency of *Staphylococcus aureus* ATCC14458.

T2-09 Transcriptional Profile of Methicillin-resistant *Staphylococcus aureus* under Different Growth Conditions

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Introduction: Historically, hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) is the predominant source for MRSA infections; however community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infections have become commonplace. Outbreaks of gastroenteritis linked to CA-MRSA contaminated ready-to-eat products have occurred.

Purpose: The purpose of this study was to sequence the genome and define the basal transcriptome of a CA-MRSA USA300 strain, an emerging foodborne pathogen, using next generation sequencing (NGS) as a preliminary step toward improved control and therapeutic strategies.

Methods: Genomic DNA was extracted from MRSA SF8300, a representative CA-MRSA USA300 strain; libraries were sequenced on the Illumina MiSeq platform (Illumina TruSeq Sample Preparation Kit; > 700,000 reads; 250bp paired-end reads). RNA was extracted from early log phase (OD_{600} = 0.4) and stationary phase (OD_{600} = 1.0 + 3hr) from MRSA SF8300 cells; libraries were quantified on the Illumina HiSeq 2500 platform in triplicate (Epicentre ScriptSeq Library Preparation Kit; > 60 million reads each; 100 bp paired-end reads). RNA-Seq reads were mapped to the MRSA TCH1516 genome sequence and normalized to fragments per kilobase per million reads (FPKM) values using the Tophat and Cufflinks packages; MULTTEST model with p-value adjustments based on False Discovery Rate (FDR) (SAS v9.3) was performed for statistical significance.

Results: Genomic DNA for strain SF8300 was assembled into 33 scaffolds with 63X coverage. Actively transcribed genes were defined as ($FPKM_{log}$/$FPKM_{stat}$ > 2; adj. *P*-value < 0.05) with 109/2,578 (4.2%) and 162/2,578 (6.2%) genes exhibiting increased transcriptional activity in log and stationary phase, respectively, over the total number of genes exhibiting activity. Major virulence gene regulator, *agr,* exhibited higher transcriptional activity in stationary phase cultures.

Significance: This study defines the log and stationary phase transcriptomes of CA-MRSA as well as the genome sequence of a typical USA300 MRSA strain. These data provide preliminary insight into methods for strain differentiation, interventions and improved therapeutics.

T2-10 Effects of Sequential Lipooligosaccharide Core Truncations on the Ability of *Campylobacter jejuni* to Attach to and Form Biofilms on Glass under Aerobic Conditions

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Introduction: *Campylobacter jejuni* is the leading cause of bacterial human foodborne gastroenteritis. The ability to attach to, and form biofilms on surfaces is important in the transmission of *C. jejuni* through food systems, leading to infections. The lipooligosaccharide (LOS) constitutes the outermost layer of *C. jejuni* cells and hence, is likely to be involved in interactions between the organism and surfaces.

Purpose: The aim of this study was to investigate if sequential LOS core truncations affect the ability of *C. jejuni* to attach to and form biofilms on glass under aerobic conditions.

Methods: LOS core of two *C. jejuni* strains, NCTC 11168 and 81-176 were sequentially truncated by inactivating genes encoding glycosyltransferases including *waaC*, *waaF, cj1135*, *cj1136*, *cj1138*, *cjj1152,* and *cjj1165*. The isogenic mutants and their wild-type were compared with respect to the numbers of attached cells, biofilm levels, autoagglutination (AAG) activity, and extracellular DNA (eDNA) production.

Results: LOS mutants exhibited increased or similar attachment, biofilm formation and AAG activity as compared to the wild-type except that two mutants lacking LOS core from glucose (11168-cj1135 and 81-176-cjj1152) showed significantly ($P < 0.05$) reduced AAG activity. There was no significant ($P > 0.05$) difference between all LOS mutants and the wild-type with respect to eDNA production. Biofilm levels significantly correlated to numbers of attached cells ($P = 0.0001$, $R^2 = 0.831$) but not to AAG activity and eDNA levels ($P > 0.343$, $R^2 < 0.100$).

Significance: These data suggest that sequential LOS core truncations may increase or not affect the ability of *C. jejuni* to attach to and form biofilms on glass, and changes in bacterial attachment due to LOS core truncations may influence biofilm formation by *C. jejuni*. These findings contribute towards a better understanding the mechanism of *C. jejuni* attachment and biofilm formation which may assist in reducing food contamination by this pathogen.

T2-11 Effect of Beef Extract Concentration on Growth of *Campylobacter* in Media Incubated Aerobically

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Introduction: *Campylobacter* is a major foodborne pathogen associated with poultry and other meats. Although *Campylobacter* are generally cultured under microaerophilic atmospheres, recent findings have indicated that this pathogen can be cultured aerobically in media containing appropriate growth factors.

Purpose: In this study, media supplemented with various concentrations of beef extract were examined for the ability to support aerobic growth of *Campylobacter*.

Methods: Basal broth composed of tryptose, yeast extract, bicarbonate, and agar was supplemented with 0.0, 2.5, 5.0, or 7.5% (wt/vol) beef extract and inoculated with approximately 10^3 CFU/ml *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni* 2b, *Campylobacter jejuni* 33560, or *Campylobacter lari*. Aliquots of inoculated media were transferred to wells of honeycomb plates, placed in a Bioscreen Microbiology Reader, and optical densities (OD) were measured during incubation for 48h at 37°C (n = 5). Inoculated media supplemented with 5.0% beef extract was also incubated aerobically in culture flasks for 72h at 37°C, and CFU/ml were enumerated after 48 and 72h incubation by plating serial dilutions of cultures on selective *Campylobacter* agar (n = 3).

Results: OD of isolates cultured in media supplemented with 2.5, 5.0, or 7.5% beef extract was significantly ($P < 0.05$) greater than OD of isolates cultured in media not supplemented with beef extract. Optimal growth of most isolates was generally produced in media supplemented with 5.0 or 7.5% beef extract. There were approximately 5 to 6 log increases in CFU/ml of *Campylobacter* recovered from inoculated media supplemented with 5% beef extract and incubated aerobically. Additionally, there was no significant difference in the number of CFU/ml recovered from supplemented media incubated for 48 or 72h.

Significance: Findings indicate that beef extract contains metabolites that may support aerobic growth of this foodborne pathogen. Use of these media may provide a less expensive method for culturing *Campylobacter*.

T2-12 Pathogens, Indicators and Antibiotic Resistance Genes in Soils with Land Applied Poultry Litter

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Introduction: Poultry litter (PL) is a by-product of broiler production most of which is land applied where it is a valuable nutrient source for crop production. PL can also be a route of contamination with manure-borne bacteria; in fact, two of the top causes of foodborne illness, *Campylobacter* sp. and *Salmonella* sp. are found in association with poultry and poultry litters.

Purpose: This two year field study was conducted to determine the fate of naturally occurring pathogens, fecal indicator bacteria (FIB) and bacteria containing antibiotic resistance genes (ARG) following application of PL to soils under conventional till (CT) or no (NT) till management.

Methods: Microbial populations were quantified in soils with applied PL using a combination of culture and quantitative, real-time (qPCR) analysis.

Results: Initial concentrations of *Campylobacter jejuni (C. jejuni)* in PL were $5.4 \pm 3.2 \times 10^6$ cells per gram PL; *Salmonella* sp. was not detected in the PL, but was enriched periodically from PL amended soils (particularly CT soils). *Escherichia coli (E. coli)* was detected in PL ($1.5 \pm 1.3 \times 10^2$ culturable or $1.5 \pm 0.3 \times 10^7$ genes per gram) but was rarely detected in field soils while enterococci ($1.5 \pm 0.5 \times 10^8$ cells per gram PL) were detected throughout the study. Within one or two days of PL application, concentrations of ARG for sulfonamide and tetracycline resistance increased orders of magnitude above background and remained elevated for the duration of the study.

Significance: These results suggest that enterococci may be better FIB for field applied PL and show that application rates (including bacterial load and nutrients) and re-application had more influence on microbial populations than did CT or NT management of soils. These data provide new knowledge about survival of important FIB, pathogens, and ARG associated with PL applied under realistic field-based conditions.

T3-01 Use of Expert Elicitation to Provide Source Attribution Estimates for the Global Burden of Foodborne Disease Initiative

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Introduction: Prevention of foodborne illness in globalized food systems requires globally comparable information on foodborne disease and its sources. The World Health Organization is coordinating a set of research initiatives that will provide the first global estimates of the burden of foodborne disease. Food source attribution estimates are an important part of the global burden of disease estimates.

Purpose: For many foodborne diseases included in the WHO Global Burden of Foodborne disease initiative, conventional observational data needed to estimates source attribution estimates was unavailable. The purpose of this study was to examine the feasibility of designing expert elicitation studies that could provide food source attribution estimates where globally comparable conventional data was unavailable.

Methods: The WHO committee commissions an expert elicitation using the Cooke classical method. This method has been applied broadly to develop parameter estimates for risk modeling where primary data is not available, but not in such a global setting. Preliminary research used review of literature, primary data, 1-on-1 interviews and pretests to evaluate alternative regionalization structures and calibration questions.

Results: The research identified a series of factors that were needed for regionalization schemes and mapped these to existing global regionalization schemes. GEMS regions were determined to be too geographically disperse and not to reflect factors other than consumption affecting source attribution. Experts involved in pretests found WHO regions too heterogeneous to be able to provide meaningful estimates. WHO sub-regions were determined to provide a balance between respondent burden and heterogeneity. Pretesting found experts were able to appropriately respond to calibration questions at the WHO sub-region level.

Significance: Preliminary design research showed that it was possible to develop an expert elicitation that could be used to in the GBD study to fill gaps in source attribution estimates.

T3-02 Food Spoilage and Safety Predictor (FSSP) Software

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Introduction: FSSP is a new and expanded version of the popular Seafood Spoilage and Safety Predictor (SSSP) software. SSSP includes models to predict seafood shelf life, histamine formation in marine fin-fish and *Listeria monocytogenes* growth and growth boundary. SSSP is extensively used to evaluate and manage growth of *L. monocytogenes* in ready-to-eat foods.

Purpose: To present new predictive models and new features included in the FSSP software to be released in 2014.

Methods: FSSP has been developed for MS Windows by using the object-oriented programming language C# and this allowed convenient addition of new models and facilities to the software. FSSP can be used in 18 different languages.

Results: New models in FSSP include:

(i) Extensive growth and growth boundary model for lactic acid bacteria (LAB) in seafood and meat products.

(ii) Expanded model to predict the simultaneous growth of LAB and *L. monocytogenes* in various seafood and meat products. This new microbial interaction model includes the effect of 12 environmental parameters and predictions can be obtained for constant or for dynamic temperature storage conditions.

(iii) Generic growth and growth boundary model. This is a simplified cardinal parameter type model that allows users to predict growth and growth boundary for any microorganism where the cardinal parameter values are known. The generic model can take into account the effect of up to 12 environmental parameters and predictions can be obtained for constant or for dynamic temperature and pH storage conditions.

Significance: The FSSP software facilitates assessment and management of microbiological risks in food and becomes available for free from http://fssp.food.dtu.dk.

T3-03 Socio-economic Determinants of Food Security in Rongo District Migori County, Kenya

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Introduction: In Kenya Rongo district is far from achieving MDG number one which is eradicating poverty and hunger despite food the security community primarily depends on local agricultural productivity and food purchasing Power. Subsistence agriculture is the main source of rural food and livelihoods. Several constraints in agriculture lead to food deficits in the region.

Purpose: The study therefore set out to determine present state of food availability and accessibility by investigating the patterns of food production, consumption, procurement and their impact on food security thus seeking to identify the environmental and social economic factors that influence food security in the district. The study recommends on strategies that can improve food security.

Methods: Research design was triangulation focusing on group discussion which was conducted to get cross sectional perspective and analyze the data collected from the interviews the target group was community leaders, agricultural extensions workers, field workers and non agricultural organization working on rural projects in the area. Nnivo 8 software was used to analyze data collected from interview and stastical package for social sciences was used for quantitative data.

Results: 70% of the land is taken by sugarcane production; maize is the most common staple food, while 90% of the respondents said maize harvest is not enough to last to the next season. The respondents cited the changing weather patterns, declining fertility of the soil, increasing pressure on land due to rapid population growth.

Significance: The findings suggest that the district suffers from lack of food which is not easily available nor accessible, affordable for the population. The study recommends the implementation of micro irrigation schemes and design and implementation of agricultural business models targeting intensive animal production.

T3-04 Critical Reflection on Assumptions of the Dutch Disease Burden Model for Food-related Pathogens

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Introduction: RIVM publishes annual disease burden estimates for 14 food-related pathogens in DALYs and cost-of-illness (COI). These estimates are used by the Ministry of Health to monitor the state, and trends therein, of microbial food safety for decision-making and policy interventions. For proper interpretation of the model outcomes, more insight into the strengths and weaknesses of the model is needed.

Purpose: To identify key model uncertainties to establish a research agenda for model improvement.

Methods: Thirteen experts with varying expertise were interviewed to identify uncertainty sources. The sources were grouped into assumption-clusters, which were scored on their scientific rigor using four so-called pedigree criteria and on the anticipated influence on model outcomes, all using a discrete 0-4 ordinal scale. Scoring was done in a structured expert elicitation workshop following the Numeral, Unit, Spread, Assessment and Pedigree (NUSAP)-approach. The scoring for the influence on end-result was done for the total DALY estimate, total COI estimate, and pathogen-ranking based on DALYs and COI. Those pathogens with low overall pedigree scores (low scientific rigor) and large influence on the outcome are key assumptions that require further study.

Results: The interviews resulted in 117 uniquely mentioned assumptions and uncertainties, grouped into 15 key assumption-clusters. The pedigree and influence scores elicited with NUSAP showed that no assumption scored highly unrigorous and/or influential. The assumption for the correction of seemingly asymptomatic shedding of pathogens in the population has been identified as the most critical among the 15 for all four outcomes. Specifically for the COI outcome, the use of GE incidence data from 1999, although corrected for pathogen specific trends, is considered a priority issue for model improvement.

Significance: Insight in priority uncertainties should be obtained in modeling studies, both for quantitative and qualitative sources. NUSAP is useful to this end to generate research agendas.

T3-05 Status of Knowledge and Implementation of Food Safety Risk Analysis Framework in Latin America and the Caribbean Region

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Introduction: Risk analysis is an important tool for modern food safety management. While risk analysis has been recommended for use in national food safety programs, the extent to which developing countries implement risk-based tools into their decision making process is unknown.

Purpose: The aim of this research was to survey government, academia, and industry sectors throughout Latin America and the Caribbean to develop an understanding of the degree of knowledge and implementation of food safety risk analysis.

Methods: A cross-sectional survey of food safety professionals in the region was designed to assess general knowledge of risk analysis, implementation in government and private industry settings, and development of risk analysis courses at universities.

Results: 279 participants from 23 countries completed the survey. Overall response rate was 67% and participation from each sector was: government (41%), academia (36%), and private (23%). Almost all the participants (97%) reported to being aware of risk analysis and 59% had received training. Most of academic institutions (83%) reported offering food safety courses with 52% covering HACCP concepts in detail and 44 and 20% covering basic principles and detailed program in risk analysis, respectively. Food companies' use of HACCP system was 35% and risk analysis 26%. Nearly all the government workers (90%) indicated the existence of national food safety regulations and 58% reported the use of risk analysis. Participants from Chile and Mexico reported to have the highest degree of risk analysis implementation. Considerable disagreement was observed among government sector in relation to the food safety regulations in place.

Significance: The survey illustrated a systemic lack of understanding of government food safety standards and implementation tools across all sectors. Food exports seemed to be connected with the higher risk analysis implementation. Additional training efforts are needed in the region to allow a fully implemented risk analysis system.

T3-06 Assessing the Potential for *Salmonella* Growth in Rehydrated Dry Dog Food in a Simulated Home Environment

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Developing Scientist Competition

Introduction: Recent outbreaks of human and canine salmonellosis linked to contamination of dry dog food and pet treats has stimulated interest in better characterizing long term survival of *Salmonella enterica* in these products. In developing an exposure assessment, it became obvious that a potentially important factor is wetting of the product by dog owners. Apparently, rehydrated pet food can remain at room temperature for substantial periods before being consumed or discarded and any substantial amplification during this period could increase exposure to both pet and owner.

Purpose: This study investigated the ability of *S. enterica* to grow in rehydrated dry dog food if held for extended periods at temperatures that would be encountered in the home.

Methods: Eight brands of dry dog food were rehydrated in measured amounts to 20, 35 and 50% moisture and inoculated with a cocktail of *Salmonella* (~10^5 CFU/g). Initial characterization examined triplicate samples incubated for 72h at 18°C, 22°C, and 28°C to simulate home environments at various seasons. Brands that supported the greatest growth were subsequently used to characterize growth kinetics.

Results: Dog food brand, moisture content, and temperature influenced pathogen growth/survival patterns. For example, at 35% moisture/28°C, 4 of 8 (50%) brands supported increases up to 3.39 log CFU/g, while levels in other four declined. Rehydration to 20% moisture generally did not support growth, while rehydration to 50%/28°C showed increases up to 4.55 log CFU/g in 5 of 8 brands. Kinetic studies at 35%/ 28°C showed growth with a calculated lag phase of 4.99h, exponential growth rate of 0.039 log cycles/ h, Tmax = 68h and Ymax = 7.77 log CFU/g.

Significance: The data provided allows rehydration of dry dog food by pet owners to be factored into the exposure assessment currently being developed for dry dog food. Observed differences among brands suggest this will be a source of substantial uncertainty and variability.

T3-07 Quantitative Microbial Risk Assessment of *Staphylococcus aureus* in Various Cheeses

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Developing Scientist Competition

Introduction: Cheeses are generally regarded as safe foods, but 0.4% of total foodborne outbreaks were linked to the consumption of contaminated cheese with foodborne pathogens in Europe.

Purpose: The objective of this study was to assess microbial risk of *Staphylococcus aureus* in various cheeses.

Methods: A quantitative microbial risk assessment for natural and processed cheeses was conducted from factory to table. In hazard identification, *S. aureus* hazard in the cheeses and microbial criteria were searched through literatures. For exposure assessment, the contamination level of *S. aureus* in cheeses was evaluated, and time and temperature distributions in cheese processing and distribution chain were also measured. Predictive

models developed in other study were used to calculate maximum specific growth rate and lag phage duration of *S. aureus*. In hazard characterization, a dose-response model for *S. aureus* was searched, and the model was used to estimate the risk of illness. With these data, in risk characterization, the risk of illness per person per day was estimated by simulation using @RISK.

Results: Monitoring results for *S. aureus* on cheeses from factory and grocery stores showed that *S. aureus* cell counts were below detection limits (0.30-0.45 log CFU/g). Thus, the prevalence of *S. aureus* on cheeses was very low. Under time and temperature distribution collected from grocery store and home, predictive models showed no significant *S. aureus* growth in natural and processed cheeses. The result of risk characterization for *S. aureus* in natural and processed cheeses showed that the mean and maximum value for the probability of illness per person a day in processed cheese were 2.24×10^{-9} and 7.97×10^{-6}, respectively, and in natural cheese, 7.84×10^{-10} and 2.32×10^{-6}, respectively.

Significance: These results should be useful in establishing the microbial regulation to control *S. aureus* in various cheeses.

T3-08 Logistic Regression Models: A Resource-focusing Tool to Identify Retail Delis with Increased Likelihood of High Prevalence *Listeria monocytogenes* Contamination

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Developing Scientist Competition

Introduction: Mitigation strategies to eliminate or reduce *Listeria monocytogenes* (LM) contamination in retail delis often require a large investment (e.g., labor, resources). Cost-effective screening tools are needed to identify and focus intervention resources in delis with the most contamination which therefore pose the greatest public health risk.

Purpose: This study developed and tested the efficacy of a model to identify retail delis with high levels of environmental LM contamination from a limited number of samples.

Methods: Forward-stepwise selection constructed a Firth's bias-corrected logistic regression model using sites from a longitudinal retail deli study (n = 30 stores, 6 months, 4508 samples). Since not all delis have floor drains yet, floor drain and drain-associated sites (e.g., floor adjacent drain) were the largest predictors (OR = 243; CI_{95}= 32, >999) in the model, a second model was constructed excluding drain-associated sites. Models were externally validated using data from the second 6-month sampling period (n = 30 stores, 4495 samples); then used to screen 50 additional delis for evidence of high LM prevalence.

Results: External validation of model A–floor drain included—successfully predicted high prevalence LM contamination in 166/180 events ($\alpha = 0.056$, $\beta = 0.022$). External validation of model B–drain-associated sites excluded—successfully predicted high prevalence LM contamination in 165/180 events ($\alpha = 0.050$, $\beta = 0.033$). We tested the models in 50 delis among 6 states. Collectively the models identified 13 of 50 delis with previously unknown LM contamination history to have increased risk for high LM prevalence; 7 stores were confirmed highly prevalent with evidence of persistence through LM environmental testing (≥2 of 20 food and non-food contact surfaces LM positive ≥2 months).

Significance: The developed screening models are conservative and potentially useful for retailer resource focusing efforts, but not recommend for use in a regulatory capacity due to the considerable false-positive identification rate.

T3-09 Evaluating Intervention Strategies to Reduce Contamination of Fresh Produce at Farm: Using Field Data to Improve the Predictive Capabilities of a "Virtual Laboratory"

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Introduction: Fresh produce can become contaminated due to contact with different contamination sources such as irrigation water, soil amendment, wild and domestic animals, and workers, among others. Given the wide range of contamination sources, there is a need to systematically characterize the contamination potential and compare the efficacy of different interventions to reduce contamination and, hence, risk of illness.

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

Methods: Pilot studies evaluated *Escherichia coli* O157:H7 contamination of romaine lettuce and *Salmonella* spp. contamination of fresh market tomatoes during the production and harvest stages. An Agent-Based Modeling framework was used to predict the contamination prevalence and levels in the growing field. Input values were derived using data from literature review, expert judgment, and data generated in a field trial experiment in California's Salinas Valley to support contamination transfer rates associated with different events (e.g., water splash from animal feces).

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

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

T3-10 Evaluation of Mathematical Models to Describe *Escherichia coli* O157:H7 Decay on Field-grown Leafy Vegetables

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Introduction: Mathematical models that predict the decay of *Escherichia coli* O157:H7 on field-grown leafy vegetables are needed for the development of accurate quantitative farm-to-fork risk assessments. The scarcity and inherent variability of data have hampered the development of suitable modeling approaches.

Purpose: To use available data to gain insight into *E. coli* O157:H7 decay patterns and to propose quantitative approaches and decay models to describe the fate of the species in field-grown leafy vegetables.

Methods: Experimental data collected from diverse sources were tabulated and both individual and pooled data sets were subjected to statistical analyses to derive mathematical models describing decay patterns using MATLAB® software. The performance of the models was challenged against independent data sets and differences between modeling approaches were analyzed by developing a probabilistic exposure assessment model to

estimate surviving *E. coli* O157:H7 populations on leafy vegetables at harvest. The probabilistic model was simulated by applying Monte-Carlo analysis implemented in @Risk®add-in for Excel.

Results: Regression analysis of individual data sets and pooled data indicated that *E. coli* O157:H7 followed a biphasic decay pattern that was satisfactorily described by the Weibull and Cerf models. Overall biphasic and monophasic models were generated that incorporated variability and uncertainty derived from the different data sets. A probabilistic exposure assessment model integrating the overall predictive models indicated that using a log-linear model (monophasic model) could lead to different risk estimates than those obtained with biphasic models. The log-linear approach yielded left-skewed distributions for surviving *E. coli* O157:H7 populations in addition to lower prevalence, and model did not account for an evident "tailing" effect in survivor curves.

Significance: This work represents the first critical assessment of the Weibull and Cerf models to describe the decay of *E. coli* O157:H7 in field-grown leafy vegetables and their suitability for microbial quantitative risk assessment.

T3-11 Study of *Escherichia coli* O157:H7 Distribution on Fresh-cut Leafy Vegetables Due to Cross-contamination during Industrial Process Simulated at Laboratory Scale

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Introduction: There is still little knowledge on the spatial pattern of *Escherichia coli* O157:H7 distribution in fresh-cut leafy vegetables where its prevalence is very low, and this information is essential to develop better sampling plans to detect pathogen contamination.

Purpose: In this work, inoculation experiments simulating real conditions were performed at laboratory scale in order to gain insight into the spread of contamination (i.e., pathogen distributions) when lettuce heads contaminated by *E. coli* O157:H7 enter a processing line during ordinary processing of fresh product.

Methods: A series of inoculation experiments at different levels of contamination were carried out in the pilot plant of National Center for Technology and Food Safety (high level, 6-7 log CFU/g; medium level, 4 log CFU/g; low level 1 log CFU/g). For each lot of production (3 kg of raw lettuce) one contaminated lettuce head was introduced, previously inoculated with a marked *E. coli* O157:H7 strain resistant to nalidixic acid. The presence and numbers of pathogen were tested in the lettuce packaged bags and washing water. Outcomes were statistically analyzed and probability distributions were fitted to count data.

Results: The study showed that *E. coli* O157:H7 is homogenously distributed on fresh-cut leafy vegetables as a result of processing (mainly washing) at both high-medium and low simulated contamination levels and this increases prevalence and spread of the pathogen over other product units. In addition, Negative Binomial, Poisson-lognormal and lognormal distributions were suitable to describe pathogen distribution at the high and medium levels, while Poisson distribution was poorly fitted to counts in spite of the Coefficient of Variation (CV) indicated no overdispersion (i.e., clustering).

Significance: These findings can have relevant implications in the development of either effective sampling plans intended to detect the pathogen at factory or other mitigation strategies to mitigate risk by *E. coli* O157:H7.

T3-12 Household Risk Mitigation Methods for Decontamination of Pesticide Residues in Tomato and Brinjal

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Introduction: The commercial production of tomato and brinjal is highly dependent on usage of different pesticides belonging to organochlorines, organophosphate, synthetic pyrethroid and neo-necotinoid groups for pest control. Farmers are not looking for the safety intervals while harvesting the crop after spray. So, these residues remain in consumed vegetables. Hence, it is essential to validate the household methods of processing for reducing pesticide residues.

Purpose: Effect of household processing methods in the removal of certain pesticides in Brinjal and tomato resulting from spray application of Profenophos 50EC@ 2ml/Lit, Chlorpyrifos 20EC@ 2ml/Lit, Dimethoate 30EC @ 4ml/Lit, Quinalphos 25EC@ 2ml/Lit, and Endosulfan 35EC 2ml/Lit. was conducted in 2012.

Methods: Samples of 10 kg were collected after 2 hours and analysed using the following mitigation methods, utilizing 1 kg for processing methods. Washing with tap water, dipping in 2% salt solution for 10 min, dipping in 2% tamarind solution for 10 min: dipping in lemon water (1 Lemon/1lit) for 10min: dipping in 0.1% sodium bicarbonate solution for 10 min: dipping in 4% Acetic acid solution for 1 min, dipping in formula 1 (4% Acetic acid+ 0.1% NAHCO3+ 1Lemon (1Lemon/1lit), cooking in pressure cooker, washing with bio wash for 10 min and control. All of the samples were replicated thrice and analysed on GC-ECD and GC-FPD for dimethoate, profenophos, chlorpyrifos, quinalphos and endosulfan residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method at the laboratory.

Results: The results show that all the treatments were effective in removing pesticide residues from Brinjal and tomato. Dipping of brinjal with 2% salt solution for 10 min was found to be the most effective treatment in removing the pesticide residues. The residues of dimethoate, chlorpyriphos, uinalphos, endosulfan and profenofos were reduced substantially using different household processing methods. In the process of running tap water washing, dimethoate residues were reduced up to 13.1%, whereas quinolphos 3.8%, endosulfan 14.1%, chlorpyriphos 16.0% and profenophos were reduced to 17.7%, but not below the MRL levels. Using direct cooking, dimethoate residues were reduced up to 74.5%, whereas the residues of chlopyriphos, quinolphos, endosulfan and profenophos were reduced to 74.5, 35.2, 37.9% and 39% respectively. In tomato, the direct cooking method has been shown to be more effective when compared with tap water washing. Washing with 2% salt water reduced dimethoate by 16.9%, and 22.2% of reduction was seen in chlorpyriphos, quinolphos (7.8%), endosulfan (9.1%) and 53.5% of profenophos. By processing with 2% salt water plus cooking, the residues from the tomato and brinjal sample were drained out from 70.6 to 92.9%.

Significance: It can be concluded that by processing with traditional processing methods, it may be possible to remove residues below MRL levels, for safer human consumption. In particular, cooking in pressure cookers removes up to 92.9%, making it very safe.

T4-01 Transfer of *Listeria monocytogenes* during Pilot-Scale Dicing of Onions

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Developing Scientist Competition

Introduction: A major nationwide recall in 2012 involving diced onions contaminated with *Listeria monocytogenes* has heightened public health concerns surrounding this product.

Purpose: Consequently, this study aimed to quantify the spread of *L. monocytogenes* during simulated commercial dicing of onions.

Methods: One 2.3 kg batch of Spanish jumbo yellow onions (*Allium cepa*) was dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) to contain ~ 4 or 2 log CFU/g, air-dried for 90 min, and then diced using an Urschel Model HA dicer, followed by ten 2.3 kg batches of uninoculated onions. Diced onion (50 g/batch) and equipment surface (100 cm^2) samples were collected after dicing, added to UVM medium, homogenized by stomaching, appropriately diluted and then plated with/without prior membrane filtration on Modified Oxford Agar to enumerate *Listeria*. All UVM-diluted samples negative by direct plating were subsequently enriched and plated. Findings from triplicate experiments were analyzed by the Tukey-Kramer HSD test using JMP 10.0.

Results: After dicing one batch of inoculated onions containing *L. monocytogenes* at 4.1 log CFU/g to contaminate the slicer followed by 10 uninoculated batches, *Listeria* was quantifiable in all samples with average populations of 3.0, 1.3, and 0.6 log CFU/g in the 1st, 5th and 10th batch, respectively. At the lower inoculation level of 2.7 log CFU/g, *L. monocytogenes* was detected by enrichment out to the 10th batch in 2 of 3 replicates. At the higher inoculation level, the bottom of the loading drum and the circular dicing blades yielded significantly ($P < 0.05$) more *Listeria* compared to other surfaces, after dicing 10 batches of onions.

Significance: These results show the ability for *Listeria* to cross-contaminate potentially large quantities of onions during mechanical dicing with such findings helping to fill one of the major knowledge gaps in risk assessments for fresh-cut produce.

Technical

T4-02 Fate of *Listeria monocytogenes* in Lettuce Wash Water during Chlorine Replenishment

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Introduction: *Listeria monocytogenes* is a foodborne pathogen frequently associated with fresh produce. For commercial fresh-cut produce wash, effective management of the chlorination process for high organic loading rates requires determination of the lower limit of free chlorine concentration necessary to control pathogenic microorganisms on produce.

Purpose: In this study, we investigated the survival of *Listeria monocytogenes* affected by the dynamic changes in water quality during the chlorine replenishment of fresh-cut lettuce wash water.

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

Results: As NaClO was incrementally added to simulated wash water, chlorination proceeded in three stages with regard to total and free chlorine concentrations. The boundaries of these stages define the combined hump and chlorination breakpoint. When the cumulative NaClO input exceeded the combined chloramine hump level and approached the breakpoint, i.e., free chlorine was approximately 3 mg/l, the *Listeria* population was reduced to an undetectable level (<0.75MPN/ml).

Significance: This study established the correlation between chlorination parameters and survival of *Listeria* monocytogenes in simulated produce wash water. It highlighted the importance of establishing performance standards for produce wash water sanitation and developing improved strategies to maintain a stable chlorine concentration during commercial produce wash operations.

T4-03 Efficacy of Non-thermal Technologies Combined with Chlorine for Reducing Microbial Populations in Ready-to-Eat Products

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Introduction: In recent years, non-thermal technologies like Ultraviolet Light and Ultrasound have shown potential as promising disinfection technologies. However, chlorine has been a widely used disinfectant in food industry. Thus, their combination could be effective on the disinfection of fresh produce.

Purpose: The purpose of the current study was to evaluate the effectiveness of the combination of two non-thermal technologies (UV and Ultrasound) with different concentrations of chlorine (50 and 200 ppm) on their ability to inactivate four different bacteria such as *Escherichia coli, Staphylococcus aureus*, *Salmonella* Enteritidis and *Listeria innocua* in three products (lettuce, strawberry and cherry tomatoes).

Methods: Commercially available romaine lettuce (*Lactuca sativa*), strawberries (*Fragaria* × *ananassa*) and cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) were purchased from a local supermarket. Experiments were conducted using four bacterial strains: *Escherichia coli* NCTC 9001, *Staphylococcus aureus* NCTC 6571, *Salmonella* Enteritidis NCTC 6676 and *Listeria innocua* NCTC 11288. The cocktail of the above microorganisms was inoculated on the three products. The treatment times selected for the non-thermal technologies were varying from 1-30 minutes followed by 3 minutes of NaOCL of two concentrations (50 ppm and 200 ppm). For the microbial analysis, ISO methods were used for the determination of the above microorganisms. A Hunterlab D25 colorimeter was used for color quality measurements.

Results: The results of the present study showed that the combination of US/NaOCL was more effective (more than 3 log reduction) in comparison with UV/NaOCL (2-3 log reduction) for reducing the cocktail of microorganisms in the above food products. Moreover, the color did not change significantly ($P < 0.05$) when short treatment times were used. Furthermore, differences in disinfection efficiency ($P < 0.05$) among the food products were obvious.

Significance: These data suggest that the combination of non-thermal with conventional disinfection technologies could find potential applications for decontamination in the food industry.

T4-04 Comparative Evaluation of Factors Affecting *Escherichia coli* Biofilms on Organic Leafy Green Wash Water Contact Surface

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Introduction: *Escherichia coli* biofilms on produce washing equipment could serve as a persistent source of cross-contamination.

Purpose: The influence of organic leafy greens wash water reuse on biofilm formation over contact surface by *Escherichia coli* O157:H7, curli-deficient *E. coli* O157:H7 and non-pathogenic *E. coli* K12 was studied.

Methods: Wash waters were prepared by washing, with and without filter sterilization, 1 batch, 6 batches and stomaching organic- baby spinach, bunched mature spinach, iceberg lettuce and romaine lettuce to simulate light, moderate and extensive reuse; and to study the influence of native produce microbiota on biofilm formation. Biofilm was allowed to develop over 48 hours at 30°C in 96-well polystyrene plates. Biofilm was quantified using Crystal Violet assay.

Results: Biofilms formed on unfiltered baby spinach wash water contact surfaces after one wash (light reuse) by *E. coli* O157:H7, curli-deficient *E. coli* O157:H7 and *E. coli* K12 resulted in OD_{600} of 0.49 ± 0.17, 0.57 ± 0.20 and 0.31 ± 0.08, respectively, while on pummeled (extensive reuse) unfiltered spinach wash water contact surface, OD600 of 1.38 ± 0.26, 2.59 ± 0.95 and 2.08 ± 0.12 resulted. On stomached filtered baby spinach wash water contact surface that was free of native microbiota, *E. coli* O157:H7, curli-deficient *E. coli* O157:H7 and *E. coli* K12 formed biofilms resulting in OD_{600} of 0.66 ± 0.18, 0.13 ± 0.12, 1.21 ± 0.47 indicating that wash water reuse and the resulting dislodged native microbiota caused synergistic biofilm formation in curli deficient *E. coli* O157:H7. On iceberg lettuce wash water contact surface containing *E. coli* O157:H7 and curli-deficient *E. coli* O157:H7, 6 washes resulted in increased biofilm formation than pummeling.

Significance: The increased biofilm formation by curli deficient *E. coli* O157:H7 with native microbiota, could indicate a strategy of environmental survival in conditions not conducive to curli expression. Curli expression but not pathogenicity seemed to play an important role in biofilm formation on produce wash water contact surfaces.

T4-05 Transgenic Plants Expressing Antimicrobial Agents for Enhancing Food Safety through Reducing Foodborne Human Pathogens in Leafy Plants

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Developing Scientist Competition

Introduction: With the increased consumption of vegetables, fresh produce is responsible for 14.8% of foodborne illness outbreaks and 22.8% of all foodborne illness cases in the United States. Conventional washing and surface sanitization cannot eliminate bacterial contamination of fresh produce, particularly for the internalized microorganisms. Facing this challenge, novel and effective intervention methods are urgently needed to improve the safety of fresh produce.

Purpose: This study provided a proof of concept that engineered plants expressing antimicrobial agent can reduce the microbial load in leafy plants.

Methods: The synthetic codon-optimized genes encoding an antimicrobial peptide, lactoferricin, were introduced into the model plant, *Arabidopsis thaliana* through *Agrobacterium*-mediated gene transformation. The integration of the transgenes into the plant genome was confirmed by PCR; mRNA transcripts of the transgenes were detected in the leaves of the transgenic plants by reverse transcriptase PCR assays. The antimicrobial activity of transgenic *Arabidopsis* was evaluated by *in vitro* and *in planta* infiltration assays. In addition, a food-grade antimicrobial protein, lactoferrin, was transiently expressed in Romaine lettuce through vacuum-infiltration with *Agrobacterium tumefaciens* carrying vectors that encodes human lactoferrin.

Results: Total protein extract from two transgenic *Arabidopsis* lines that expressed lactoferricin reduced the *Escherichia coli* population (inoculum, 6.5 logs) by 86% and 78%, respectively, in the *in vitro* activity tests. Compared to the wildtype plants, three independent transgenic *Arabidopsis* lines expressing lactoferricin significantly decreased the infiltrated bacterial population (inoculum level, 4.5 logs) in leaves by 63%. The transient expression level of lactoferrin in lettuce was 57.8 ng/g lettuce tissue as determined by ELISA.

Significance: This is the first report of transgenic *Arabidopsis* with increased resistance to a foodborne human pathogen. The study suggested that transgenic technology is promising to promote the microbiological safety of fresh produce. Transgenic lettuce plants expressing antimicrobial agents for enhancing food safety are being developed in our group.

T4-06 *Salmonella* Attachment and Biofilm Formation on Tomatoes and Equipment Surfaces as Impacted by Organic Load, pH and Temperature

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Developing Scientist Competition

Introduction: Bacterial attachment and biofilm formation on tomatoes and processing equipment surfaces is impacted by multiple intrinsic and extrinsic parameters.

Purpose: This study assessed the impact of two substrates and three temperatures on *Salmonella* attachment and biofilm formation on tomatoes as well as stainless steel and HDPE surfaces found in processing facilities.

Methods: Changes in viability, morphology, surface hydrophobicity and surface charge were assessed in diluted trypticase soy broth + yeast extract (pH 4.6 or 7.0) that was inoculated to contain avirulent *Salmonella* Typhimurium LT2 at ~9 log CFU/ml and held for 8 days at 4, 10, and 23°C. Attachment and biofilm formation as determined by direct plating and/or Confocal Scanning Laser Microscopy (CSLM) was also assessed on three surfaces of different hydrophobicities (contact angles) – tomatoes (100), high-density polyethylene (HDPE) (63.1), and stainless steel (35.6) that were inoculated with two substrates – water or 10% (w/v) blended tomatoes containing ~8 log CFU/ml *Salmonella* and then held for 6 days at 4, 10 and 23°C.

Results: *Salmonella* viability remained constant with cell elongation observed after 8 days of incubation at 4°C. *Salmonella* surface charge was higher at pH 4.6 with hydrophobicity inversely related to temperature. Significantly greater ($P < 0.05$) attachment to tomatoes was seen at 4°C using the blended tomato substrate. Biofilm formation significantly decreased ($P < 0.05$) with temperature for both substrates. Water as a substrate led to greater ($P < 0.05$) attachment and biofilm formation on HDPE at 23°C and on stainless steel at 23 and 10°C. Confocal imaging showed more uniform distribution of *Salmonella* on stainless steel as opposed to HDPE and tomatoes.

Significance: Based on these findings, tomato storage < 10°C and processing in water having a minimal organic load and an acidic pH is recommended to decrease *Salmonella* attachment and biofilm formation, with sanitizer efficacy also enhanced under these same conditions.

T4-07 Identification of *Staphylococcus aureus* Genes Expressed for Acid Stress Response in Tomato

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Developing Scientist Competi

Introduction: *Staphylococcus aureus* is a foodborne pathogen, and the pathogen has occasionally been isolated from tomatoes. However, the strain variation of *S. aureus* has been shown in acidic environments.

Purpose: The objective of this study was to evaluate acid resistance of *S. aureus* strains and to identify genes related to acid tolerance of *S. aureus* in tomatoes.

Methods: *S. aureus* strains ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826 were habituated in tomato extract at 35°C for 24h, and *S. aureus* cells were then harvested by centrifugation. The cells of tomato-habituated *S. aureus* strains and non-habituated *S. aureus* strains were then exposed to tryptic soy broth adjusted to pH 3.0 for 60 min, and survivals of the strains were enumerated on tryptic soy agar. Total RNA of tomato-habituated (15 and 60 min) and non-habituated *S. aureus* strains were extracted, and cDNA was then synthesized to evaluate the expression level of acid-shock related genes by quantitative real-time PCR.

Results: After acid challenge, *S. aureus* ATCC23235 showed the most obvious difference in survival between tomato-habituated and non-habituated *S. aureus*, and *S. aureus* ATCC14458 was the second most resistant. Four genes (*clpB*, *zwf*, *nuoF*, and *gnd*) were selected as acid-shock related genes from tomato-habituated and non-habituated *S. aureus*, and there was no obvious difference in gene expression levels after 15-min tomato-habituation. However, tomato-habituated *S. aureus* ATCC23235 showed higher expression of *clpB*, *zwf*, *nuoF*, and *gnd* genes after 60 min, compared to non-habituated cells.

Significance: These results indicate that different *S. aureus* strains have different acid stress response in tomato due to higher expression level of *clp*, *zwf*, *nuoF*, and *gnd* gene.

Technical

T4-08 Selection of Index and Surrogate Bacteria for Validation and Verification of Combined Thermal and Oxidizer Inactivation of Pathogens Associated with Cantaloupe Rind

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Developing Scientist Competition

Introduction: Validation of process controls often requires surrogates that closely match the tolerance traits of stress-adapted contaminants.

Purpose: Surrogate and pathogenic bacteria were evaluated for survival at thermal exposure shown to be non-injurious to whole cantaloupe.

Methods: Seven cantaloupes were washed in 4 l of tap water and 200 ml was heated to 95 or 55°C alone or with peroxyacetic acid (PAA) (20, 50, and 80 ppm) or lactic acid (LA) (0.5, 1, and 2%). After 0.5 to 30 min, samples were quantified for total heterotrophs and total coliforms using Plate Count Agar and Quanti-Tray, respectively. 9 strains of *Salmonella*, 2 *Listeria* spp., 6 strains of *Escherichia coli*, or 1 strain of *Pseudomonas fluorescens* were inoculated onto detached cantaloupe periderm and, after air-drying, contained ~4-8 log CFU/g. 0.1-g were added to 9 ml of 70°C Trypticase Soy Broth (TSB) and held for 0.25 to 30 min before rapid cooling. Samples were quantified or enriched to determine presence.

Results: 95°C exposure for 2 minutes (no oxidizer) resulted in 3.6 and > 6.0 log reductions of indigenous heterotrophic bacteria and total coliforms. 95°C and 55°C water with 80 or 50 ppm PAA were significantly more effective ($P < 0.05$) at reducing populations of total heterotrophs after 2 min (avg. 3.60 log CFU/ml) than other treatments. After 30 min, 95°C and 55°C with 80 ppm PAA were more effective ($P < 0.05$) at reducing total heterotrophs than 55°C + 2% LA; all four treatments achieved a 6-log reduction of total coliforms. Four strains survived 30 min at 70°C – *L. monocytogenes*, *L. innocua*, *Salmonella* Cubana, and *Salmonella* Senftenberg.

Significance: Heated oxidizers during cantaloupe washing enhance reduction of indigenous bacteria used as an index for process verification. Additionally, these results delineate the importance of proper challenge strain selection when assessing thermal inactivation treatments.

T4-09 Chlorine Dioxide Gas Treatment of Cantaloupes and Quantification of Its Residues

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Developing Scientist Competition

Introduction: In previous studies, cantaloupes treated with chlorine dioxide (ClO_2) gas (5 mg/l, 10 min) showed 4.6 and 4.3 log reductions of *Escherichia coli* O157:H7 and *Listeria monocytogenes*, respectively. This could however result in residues of ClO_2 specifically, chloride (Cl^-), chlorite (ClO_2^-), chlorate (ClO_3^-) and perchlorate (ClO_4^-) in the treated product, of which, the latter three are a toxicity concern.

Purpose: The purpose of this study was to treat cantaloupes with ClO_2 gas (5 mg/l, 10 min) and then identify and quantify its residues.

Methods: Radiolabeled chlorine dioxide ($^{36}ClO_2$) gas was generated by an acidification reaction and used to treat a cantaloupe in six individual treatments. Cantaloupe flesh (~200 g) was also directly exposed to $^{36}ClO_2$ remaining in the system after each of the last three treatments. Each treated cantaloupe was separated into rind, flesh and mixed (rind+flesh) samples. These were blended to give the corresponding 'slurry'. Aliquots of slurry were centrifuged to obtain the supernatant 'serum'. Serum aliquots were fractionated via ion chromatography and the fractions collected. Liquid scintillation counting was used to detect radioactivity in collected fractions, aliquots of serum and aliquots of slurry. Radioactivity detected and the ratio of radioactivity to ClO_2^- mass for the generation of $^{36}ClO_2$ gas was used to calculate residue concentrations.

Results: Anions detected in the cantaloupe were Cl^-(~90%) and ClO_3^-(~10%). They were located primarily in the rind (19.26 ± 7.99 µg Cl^-/g rind slurry and 4.83 ± 2.26 µg ClO_3^-/g rind slurry, n = 6). Only Cl^- residues (8.12 ± 1.01 µg Cl^-/ g flesh, n = 3) were detected in cantaloupe flesh directly exposed to ClO_2 gas.

Significance: Transference of these residues from rind to flesh may occur during cutting, however, Cl^- is not considered toxic and ClO_3^- exposure is too low to be a cause for concern. This data suggests therefore that ClO_2 residues in cantaloupes do not pose a significant toxicological risk.

T4-10 Effectiveness of Broad Spectrum Chemical Intervention Treatments against *Escherichia coli* O157:H7, Non-O157 STEC, *Listeria innocua*, and *Salmonella* on Artificially Inoculated Cantaloupe and Watermelon

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Introduction: The presence of several foodborne pathogens including *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in outbreaks linked to cantaloupe and watermelon have raised concern over how to control these outbreaks in the melon industry. There is a need for identification of a broad spectrum post-harvest intervention treatment effective for melons with varying surface types.

Purpose: An experiment was conducted to evaluate the effectiveness of six different categories of commercially available produce washes to determine the most effective ones against a broad spectrum of foodborne pathogens on cantaloupe and watermelon.

Methods: The produce wash (sodium hypochlorite, hydrogen peroxide, liquid chlorine dioxide, peroxyacetic acid/hydrogen peroxide combinations, peroxyacetic acid/acetic acid/hydrogen peroxide combination, organic acids, and quaternary ammonium) were tested against: *E. coli* O157:H7, *Listeria innocua*, *Salmonella* spp., and a cocktail of Big 6 *E. coli* non-O157 STEC artificially inoculated on the surface of cantaloupe and watermelon. Surface treatments were applied to each melon type and reductions of pathogens were statistically determined with SAS.

Results: There were significant differences observed between produce washes, but not between melon types ($P < 0.05$). For both types of melons, the three most effective sanitizers observed were quaternary ammonium, peroxyacetic acid/hydrogen peroxide combination, and the peroxyacetic acid/acetic acid/hydrogen peroxide combination with 1.0 - 2.2 log CFU/g, 1.3 - 2.6 log CFU/g, and 1.3 - 3.5 log CFU/g reductions, respectively, on all tested pathogens. Additional tested washes were less consistent, with reductions ranging from 0-3 log CFU/g depending on pathogen type.

Significance: Based on study results, we determine that quaternary ammonium and peroxyacetic acid/hydrogen peroxide products were most effective against the foodborne pathogens of concern on the surface of cantaloupe and watermelon. This study is of interest to public health personnel and the fruits and vegetable industry, for the control of these pathogens in the food supply.

T4-11 Biocontrol of *Listeria monocytogenes* and *Escherichia coli* O104:H4 in Fresh Cut Cantaloupe, Alfalfa Seeds and Sprouts Using Immobilized Bacteriophages

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Introduction: Due to lack of adequate control methods to prevent contamination in fresh produce and growing consumer demand for natural products, the use of bacteriophages has emerged as a promising approach to enhance safety of these food products.

Purpose: The objective of this research is to control the growth of *Listeria monocytogenes* and *Escherichia coli* O104:H4 in contaminated cantaloupes, alfalfa seeds and sprouts respectively, under different storage conditions by using specific non-immobilized and immobilized lytic bacteriophage cocktails.

Methods: Bacteriophage cocktails were immobilized either on positively charged modified cellulose membranes or encapsulated in alginate beads. Phage-treated and non-treated samples were stored for various incubation period and temperatures.

Results: In cantaloupe, when phage cocktail was added in the non-immobilized format, the growth was below the detection limit of the plating technique (< 1 log CFU/gm) after 5 days of storage at 4°C and 12°C. While at 25°C, the growth was below the detection limit after 3 and 6 hours and a 2-log CFU/gm reduction was observed after 24 hours. For the immobilized phage experiment, around 1-log CFU/gm reduction in the bacterial count was observed by the end of the storage period for all tested storage temperatures. For the alfalfa seeds and sprouts experiments, regardless of the phage application technique, sprayed, encapsulated, or impregnated, the growth of *E. coli* O104:H4 was below the detection limit after 1 hour in seeds and around 1-log reduction in bacterial count was observed on the germinated sprouts by day 5.

Significance: Phage, non-immobilized or immobilized, can be considered as a promising tool to enhance food safety in fresh produce.

T4-12 Use of Bacteroidales Source-tracking Markers to Evaluate the Effect of Hand Washing or Sanitizing on Fecal Contamination of Produce Workers' Hands

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Introduction: In an earlier study, we investigated the utility of Bacteroidales 16S rDNA source-tracking for identifying fecal contamination, and its source, in the fresh produce production environment. The method was applied to rinses of fresh produce, source and irrigation waters, and harvester hand rinses from farms in Northern Mexico, and results suggested that farm workers' hands were a risk factor for fecal contamination of produce.

Purpose: As a proxy for fecal contamination, the purpose of this study was to determine if hand hygiene interventions have immediate and/or residual effects on the levels of general and source-specific Bacteroidales on produce workers' hands.

Methods: The hand rinses of 158 Jalapeño farm workers were collected during the summer of 2013. Workers were divided into 3 intervention groups, control (40), hand washing (60), and sanitizing (60) groups; the latter two of which were subdivided into groups for which samples were collected pre-intervention (20) or post-intervention (40). Bacteria were concentrated from hand rinse samples using a combined centrifugation/filtration method and the DNA extracted using the MP Bio FastSpin kit for soil. Quantification of the universal and human-specific Bacteroidales markers was performed using the AllBac and BFD primers and probes, respectively.

Results: The AllBac marker was detected in 81% (123) of samples, with a geometric mean concentration of 1.6 log genome equivalent copies (GEC) per 100 ml hand rinse sample. The human marker was identified in 46% (69) of samples, with a geometric mean concentration of 2.6 log GEC per 100 ml. There was no significant difference in the AllBac or BFD marker concentrations between intervention groups ($P > 0.05$).

Significance: Neither intervention had an immediate or a residual effect on the levels of general and source-specific Bacteroidales on produce workers' hands, suggesting little impact of the intervention on fecal contamination.

T5-01 Increasing Number and Greater Morbidity and Mortality Associated with Multistate Foodborne Disease Outbreaks — United States, 1973–2010

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Introduction: Approximately 800 foodborne disease outbreaks are reported in the United States each year. Multistate foodborne disease outbreaks can be an important source of information about foods contaminated during food production and distribution.

Purpose: Describe the frequency and characteristics of multistate foodborne disease outbreaks.

Methods: We reviewed outbreaks reported to CDC's Foodborne Disease Outbreak Surveillance System from 1973 through 2010. Multistate foodborne disease outbreaks were defined as ≥2 persons in multiple states with similar illnesses after exposure to a common food. We analyzed the number of illnesses, hospitalizations, and deaths; pathogens; and implicated foods.

Results: From 1973 through 2010, multistate foodborne disease outbreaks accounted for 234 (0.8%) of 27,989 total outbreaks and resulted in 3% of all outbreak-associated illnesses, 10% of hospitalizations, and 16% of deaths. On average, 2.9 multistate outbreaks occurred annually during 1973–1980, 1.4 during 1981–1990, 6.3 during 1991–2000, and 13.4 during 2001–2010. Among 230 outbreaks with a pathogen reported, most were caused by *Salmonella* (109; 47%) and Shiga toxin-producing *Escherichia coli* (60; 26%). In 174 outbreaks (74%), a food was reported and could be classified into one of 18 single food commodities; the commodities most commonly implicated were beef (39, 22%), fruits (23, 13%), and leafy vegetables (22, 13%).

Significance: From 1973 through 2010, multistate outbreaks accounted for an increasing number of foodborne disease outbreaks. Although multistate outbreaks represent only a small proportion of all outbreaks, they are responsible for a greater share of outbreak-associated hospitalizations and deaths than single state outbreaks. Knowing the pathogens and foods responsible for multistate outbreaks can help to identify important sources of food contamination and inform regulatory agencies and food industry partners to improve the safety of the food supply.

T5-02 Foodborne Disease Outbreaks Associated with Organic Foods — United States, 1973–2012

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Introduction: Organic food sales have increased steadily over the last decade, from $11 billion in 2004 to $27 billion in 2012. Organic foods are often credited as providing health benefits; however, data surrounding food safety considerations are lacking.

Purpose: To describe the frequency of outbreaks caused by organic foods in the United States.

Methods: We reviewed outbreaks reported to CDC's Foodborne Disease Outbreak Surveillance System during 1973-2012 where the implicated food was reported to be organic. We also searched the internet for unreported outbreaks. Data analyzed included number of outbreaks, etiologic agents, and implicated foods.

Results: From 1973-2012, 15 outbreaks associated with organic foods were identified, resulting in 577 illnesses, 182 hospitalizations, and 3 deaths. The first identified outbreak with an organic food occurred in 1992; 47% of the outbreaks occurred during 2010-2012. Nine single state outbreaks were reported from five states (California, 3 outbreaks; Minnesota, 3; Maine, 1; Florida, 1; Michigan, 1); six multistate outbreaks had cases from 37 states and the District of Columbia. Outbreak etiologies were *Escherichia coli* O157:H7 (6 outbreaks, 40%), *Salmonella* (6, 40%), *Campylobacter* (1, 7%), and *Clostridium botulinum* (1, 7%). One outbreak involved multiple etiologies (*Escherichia coli* O157:H7, *Campylobacter*, and *Cryptosporidium)*. Seven outbreaks were attributed to produce (carrot juice, spinach, spring mix, alfalfa sprouts, salad mix, grape tomatoes, and an unspecified produce item), four to unpasteurized dairy products, two to eggs, and two to multi-ingredient foods.

Significance: Although infrequent, organic foods have been implicated in outbreaks and more outbreaks were reported in recent years. These findings might underestimate the number of outbreaks due to organic foods because the origin of many foods causing outbreaks is not known or reported. Efforts to improve the safety of the food supply should include gathering better data on the origin of implicated foods, including growing and production methods.

T5-03 Monitoring Trends in Foodborne Disease Using U.S. Poison Center Data: 2000–2011

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Introduction: Surveillance of foodborne disease (FBD) is a critical part of the food safety system and provides important information that is used to detect outbreaks, monitor trends, quantify disease burden, identify food safety hazards and potential interventions, and set public health priorities. Consumer complaint data have become increasingly useful for FBD surveillance and detection of outbreaks. Despite potential benefits, few studies have evaluated the effectiveness of syndromic surveillance for FBD.

Purpose: A retrospective analysis of self-identified FBD exposures reported to U.S. Poison Centers between 2000 and 2011 was undertaken to explore the use of National Poison Data System (NPDS) as an early detection surveillance system for FBD.

Methods: Descriptive statistics were used to characterize NPDS self-reported FBD exposures, including gender, age, caller location, product implicated, exposure reason, exposure site, exposure duration, clinical effects, duration of clinical effects, and medical outcome, and analyze trends in case prevalence over time. CDC's Early Aberration Reporting System was used to identify anomalies in daily case prevalence. The number of anomalies in case prevalence identified within 14 days before or after outbreaks and recalls were tabulated.

Results: There were 433,788 unique, self-reported cases of suspected FBD from 2001-2011. Overall, there was a decreasing trend in case prevalence. Only about half of cases reported common gastrointestinal symptoms. Eight anomaly-events in case prevalence occurred within 14 days before or after national outbreaks or recalls; seven of these occurred after a specific outbreak or recall.

Significance: These data suggest that NPDS is a potential source of complaint data that could be used to monitor FBD. Several limitations, including questionable reliability of self-reported FBD cases, affect the utility of NPDS data. However, improved data collection and coordination with public health agencies may improve the ability to use NPDS data to monitor FBD, identify potential outbreaks, and improve situational awareness.

T5-04 Estimates of Foodborne Illness Hospitalizations and Deaths in Canada

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Introduction: Foodborne illness estimates help set food safety priorities and create public health policies. In 2013, the Public Health Agency of Canada estimated that 4 million episodes of foodborne illness occur each year in Canada due to 30 known pathogens and unspecified agents. As follow up to this work, the Public Health Agency of Canada has estimated the number of hospitalizations and deaths.

Purpose: There were two overall objectives: (1) to estimate the number of domestically acquired foodborne illness related hospitalizations and deaths and (2) identify knowledge gaps for further research.

Methods: Using the 2013 estimates of foodborne illness for Canada along with data from the Canadian Hospitalization Morbidity Database (for years 2000-2010), relevant international literature and the 2006 Canadian census population estimates of the number of hospitalization and deaths

for each pathogen and unspecified agents were calculated. The analysis accounted for under-reporting and under-diagnosis. Estimates on the proportion foodborne and the proportion travel-related were incorporated for each pathogen. Monte Carlo simulations were performed to account for uncertainty using @Risk software generating mean estimates and 90% credible intervals.

Results: There are an estimated 4,700 hospitalizations and 150 deaths related to domestically acquired, foodborne illness due to 30 pathogens each year in Canada. Key pathogens associated with these hospitalizations and deaths include norovirus, non-typhoidal *Salmonella* spp, *Escherichia coli* O157 and *Listeria monocytogenes*.

Significance: This is the first time Canada has pathogen-specific estimates of domestically acquired foodborne illness related hospitalizations and deaths. This information illustrates the substantial burden of foodborne illness in Canada. Policy makers, industry, academia and other organizations can use these estimates to better inform policy, research, food safety risk assessments, education campaigns and other prevention and control activities – ultimately improving the health of Canadians.

T5-05 Epidemiologic Attribution of Foodborne Norovirus Outbreaks, United States, 2009–2012

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Introduction: Noroviruses are the leading cause of both sporadic cases and outbreaks of foodborne illness in the United States. Attribution of foodborne norovirus disease is critical for development of prevention strategies and relies primarily on outbreak surveillance data. Implementation of the National Outbreak Reporting System (NORS) in 2009 included changes to how national foodborne outbreak data are collected, providing opportunity for an updated analysis of foodborne norovirus outbreaks.

Purpose: Describe foodborne norovirus outbreaks in the United States reported through NORS and characterize their attribution by setting, contributing factors, and implicated foods.

Methods: Data reported to the Centers for Disease Control and Prevention (CDC) through NORS for all suspected and confirmed foodborne norovirus outbreaks during 2009–2012 were extracted. Descriptive frequencies of outbreak characteristics were generated using standardized categorization schemes.

Results: During 2009–2012, 1,018 foodborne norovirus outbreaks were reported to CDC, resulting in 21,320 reported illnesses (median 12 illnesses/outbreak, range 2–303), 1,141 healthcare provider visits, 536 emergency department visits, 217 hospitalizations, and 2 deaths. Norovirus was laboratory confirmed as the etiology in 664 (65%) outbreaks; most (82%) were caused by genogroup II, specifically genotype GII.4 (53%). Restaurants were the most frequently reported setting of food preparation (60%), followed by catering or banquet facilities (18%) and private homes (7%). Among the 496 (49%) outbreaks in which factors contributing to contamination were reported, a food worker was implicated as the source of contamination in 373 (75%). At least one food was implicated in 380 (37%) outbreaks. Most implicated foods were consumed raw (70%) and considered ready-to-eat (57%); salads (23%) and sandwiches (7%) were implicated most frequently.

Significance: Foodborne norovirus outbreaks continue to occur with great frequency in the United States and result in substantial morbidity. Prevention efforts should target appropriate practices for handling of ready-to-eat foods and hygiene of food workers.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

T5-06 Food Safety Aspects of Emerging Zoonotic Viruses: The Case of Avian Influenza H7N9 and the Middle East Respiratory Syndrom (MERS-CoV)

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Introduction: 2012 and 2013 have seen the emergence of 2 new pathogens of concern: H7N9 and MERS-CoV. H7N9 is a new avian influenza virus severely affecting humans and with a substantial mortality rate. While the exact exposure leading to infection is not known, it is believed that close contact with poultry especially in poultry market is a main source of infection. MERS-CoV is an emerging coronavirus of zoonotic origin causing severe disease in humans. So far, cases of H7N9 are linked to exposure in China and MERS-CoV are linked to exposure in the Middle East.

Purpose: The presentation will describe these 2 emerging zoonotic diseases and will discuss their link to exposure to food producing animals and their potential food safety implications.

Methods: Up to March 2014, approx. 390 human cases of H7N9 and approx. 200 cases of human MERS-CoV have been reported. Understanding their different possible exposures to contaminated environment, infected animals or their products can give information on the possible food safety implication of these 2 diseases and on possible ways of mitigating their further spread.

Results: H7N9 is an influenza virus of avian origin and has been detected in poultry in different affected provinces in China. The virus has also been detected in live bird markets where human cases have reported having contact with poultry or their environment. MERS-CoV has been detected in dromedary camels. Other livestock possibly implicated are also being investigated. A substantial number of human cases of MER-CoV infections have reported contacts with camels or with livestock or their products.

Significance: The possible confirmation of these common livestock as an important source of exposure for humans to these 2 viruses could pose serious challenges in terms of prevention and control, especially if the viruses do not cause obvious disease or symptoms in livestock animals. Based on our current understanding of the persistence of these viruses in the environment, animals and their food products, the presentation will discuss their potential impact in terms of food safety and food handling.

T5-07 Prevalence and Characterization of *Salmonella* spp. Isolated from Feral Pigs in Texas

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Developing Scientist Competition

Introduction: While it is well established that livestock are a key reservoir for *Salmonella*, the importance of wildlife species as reservoirs for this zoonotic foodborne pathogen is poorly defined. The population of feral pigs (*Sus scrofa*) in the United States might be as high as 8 million, with an estimated 2.6 million in Texas alone. Feral pigs are known to invade agricultural lands in search of food, which has led to the dissemination of zoonotic enteric pathogens through fecal contamination of crops in the past.

Purpose: Our aims were to determine the prevalence of *Salmonella* among feral pigs in Texas and to characterize the antimicrobial susceptibility of these isolates, thus facilitating an assessment of public health risk presented by this wildlife reservoir.

Methods: We have an ongoing active surveillance program for *Salmonella* fecal shedding among feral pigs in Texas. This program is supported by a collaboration with USDA-APHIS-Wildlife Services, which has a dedicated system in place for managing feral pig damage and disease concerns throughout the state. Fecal samples are being sent to Texas A&M University for *Salmonella* isolation using standard bacteriologic culture techniques. Antimicrobial susceptibility testing is performed on isolates using the Sensititre broth microdilution method. Date, geographic location, and data on each pig are collected.

Results: To date, fecal samples have been obtained from 99 feral pigs in 14 counties throughout Texas. Twenty-four (24.2%) samples from 10 counties were culture-positive for *Salmonella* spp. Those *Salmonella* isolates (n = 17) tested against a panel of 15 antimicrobial agents were pansusceptible.

Significance: This ongoing study is one of the first to shed light on the ecology and epidemiology of *Salmonella* among feral pigs in the U.S. Our data indicate that *Salmonella* is frequently found among feral pigs in Texas, with important implications for public health.

T5-08 Prevalence and Characterization of *Escherichia coli* O104 in Cattle Feces

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Developing Scientist Competition

Introduction: The serotype O104:H4 reported in 2011 outbreak is a hybrid strain, which possesses characteristics of two pathotypes, Shiga toxin-producing and enteroaggregative *Escherichia coli*. Although O104:H4 has not been detected in cattle, but other serotypes such as O104:H21, O104:H2, O104:H7, O104:H12 and O104:H16 have been detected in cattle feces.

Purpose: To estimate the prevalence of *E. coli* O104 in feedlot cattle feces and characterize the isolated strains.

Methods: A total of 757 cattle rectal content samples, representing 29 different feedyards, were collected at a slaughter plant. Fecal samples were enriched in *E. coli* broth for 6 hrs at 40°C. DNA extracted from pre and post enriched fecal samples were tested by a multiplex PCR to detect serogroup O104 and associated virulence genes of the hybrid strain. Post-enriched fecal samples were also subjected to culture-based method of detection, which involved immunomagnetic separation with O104 beads, plating on selective chromogenic medium, followed by serogroup confirmation by PCR of pooled and individual colonies. Pure cultures of O104 were characterized by multiplex PCR assays to determine virulence genes and flagellar types.

Results: Of the 757 samples, 38 (5%) were positive before enrichment and 349 were positive (46%) after enrichment of fecal samples for O104 serogroup specific gene. We obtained 143 O104 isolates by culture-based method of detection, of which 65 of them were positive for *terD*, 16 were positive for *hlyA* and 16 for *stx1*genes. O104 isolates harbored diverse flagellar (H) antigens, 45 isolates were positive for H21, 38 for H11, 37 for H7 and 5 for H2 antigens. Ninety-two of the isolates were also found positive for O8/O9 antigen genes.

Significance: Cattle harbor and shed *E. coli* O104 in feces, but only a few strains (11.18 %) carried *stx*1 gene. None of the isolated strains carried genes characteristic of enteroaggregative type.

T5-09 Whole Genome Sequencing-based Benchmarking of Subtyping Methods for *Salmonella enterica* Serotype Enteritidis

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Introduction: Powered by whole genome sequencing (WGS) technologies, recent implementations of whole genome single nucleotide polymorphism typing (WGST) have led to significant improvements of both molecular subtyping and phylogenetic analyses, particularly for monophyletic bacterial pathogens. WGST allows discovery of SNPs across the entire bacterial genomes, therefore, provides superior subtyping resolution and phylogenetic accuracy, which make it ideal for benchmarking other subtyping methods.

Purpose: To compare the respective performance of commonly used and newly developed subtyping methods in delineating each individual outbreak under the guidance of the recently proposed phylogenetic framework and population structure of commonly circulating SE lineages in the United States.

Methods: A cohort of 52 *Salmonella enterica* serotype Enteritidis (SE) isolates representing 16 major outbreaks and three sporadic cases in the United States between 2001 and 2011 were sequenced and subject to a retrospective investigation in combination with four subtyping methods.

Results: Whole genome single nucleotide polymorphism typing (WGST) resolved all outbreak clusters and provided useful epidemiological information with robust phylogenetic inference. While both MLVA and CRISPR-MVLST yielded higher discriminatory power than PFGE, MLVA outperformed in delineating outbreak clusters whereas CRISPR-MVLST showed the potential to cluster major lineages and ecological origins of SE.

Significance: Our results suggested that whole genome sequencing (WGS) based approaches make a viable platform for the evaluation and benchmarking of other molecular subtyping methods.

T5-10 Methicillin-resistant Coagulase-negative Staphylococci (MRCoNS) in Retail Meat

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Introduction: Coagulase-negative Staphylococci (CoNS) have been demonstrated as a larger reservoir of *mecA* than *Staphylococcus aureus* and speculated as the origin of *mecA*. Since methicillin-resistant *S. aureus* (MRSA) has been recovered from US retail meat, the extent of methicillin-resistant coagulase-negative staphylococci (MRCoNS) in retail meat needs to be studied.

Purpose: To understand the extent of MRCoNS serving as the *mecA* reservoir in retail meat.

Methods: MRCoNS were isolated from retail meat (beef, chicken, and turkey) in Detroit and characterized by *sodA* gene sequencing for species identification, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and pulsed-field gel electrophoresis (PFGE).

Results: Unique MRCoNS isolates recovered from 25 meat samples were comprised of *S. sciuri* (n = 13), *S. fleuretti* (n = 4), *S. lentus* (n = 3), *S. epidermidis* (n = 2), *S. vitulinus* (n = 1), *S. saprophyticus* (n = 1) and *S. pasteuri* (n = 1). Heterogeneous and composite SCC*mec* types, including I, III, IV, V, I+V and III+V were identified in 16 isolates. Same SCC*mec* types were recovered in different staphylococcal species and meat sources. Indistinguishable PFGE patterns were also observed in *S. sciuri* isolated from beef, chicken, and turkey, and with different SCC*mec* types.

Significance: Multiple CoNS species can serve as reservoirs for *mecA*. In addition to the clonal transmission of MRCoNS in meat, horizontal occurrence of SCC*mec* is observed in staphylococcal species.

T5-11 Genetic Relatedness of *Escherichia coli* and Male-specific Coliphages' Fecal Contamination Sources in Salads from Corporate and Locally Owned Restaurants

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Introduction: Food workers are responsible for approximately 20% of foodborne outbreaks and so there is a pressing need for the restaurant industry to identify the potential vulnerabilities in their practices.

Purpose: The underlying hypotheses were that genetically related *Escherichia coli* will be present in restaurant salads over multiple sampling frames and that male-specific coliphages indicative of human and animal waste could be isolated from restaurant salads.

Methods: The focus of this study was to understand the genetic relatedness of *E. coli* isolates and male-specific coliphage isolates obtained from 200 salad samples obtained from 5 corporate owned and 5 locally-owned restaurants from a university town between September 2009 and May 2010.

Results: Out of 200 samples, 40 samples (20%) tested positive for male-specific coliphages, and 37 samples (19%) were positive for *E. coli*. *E. coli* isolates were DNA fingerprinted using the Diversilab® DNA fingerprinting into genotypes, while male-specific coliphages were genogrouped using RT-PCR. Genogrouping of phages into genogroups I, II and III allowed source (animal or human) identification. Both salad types from both restaurant types were positive for genogroups I (animal waste) and III (human waste). The genogrouping revealed that salad samples were being exposed to both human and animal fecal contamination. Samples from multiple time points were positive. Four distinct *E. coli* genotypes were detectable. Identical genotypes were detected in samples obtained from multiple time points suggesting the persistence of *E. coli* genotypes in some restaurants, or of a common contamination source in the produce supplies. *E. coli* genotyping revealed the same genotype at multiple restaurants suggesting a common contamination point somewhere between the growing areas and the retail/wholesale distribution.

Significance: These results highlight the importance of screening for multiple fecal contamination indicators, and fingerprinting tools to identify contamination sources. The importance of disinfection of salad ingredients within restaurants is also highlighted.

T5-12 Preliminary Results of a National Survey of Local Health Departments Designed to Examine Restaurant-related Foodborne Illness Outbreaks, Restaurant Inspections and Food Handler Training

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Developing Scientist Competition

Introduction: A considerable proportion of foodborne outbreaks in the United States are attributed to restaurants. Ensuring food safety among food establishments is an important feature of local health department efforts to protect public health.

Purpose: The purpose of this study is to determine if frequency of restaurant foodborne outbreaks is associated with restaurant inspection frequency and levels of food handler training when controlling for local demographic factors.

Methods: To obtain local restaurant-related data, each of the 2,900 local US health departments were contacted and invited to complete a web-based survey. The 50-question survey instrument was designed to collect jurisdictional demographics, frequency of foodborne outbreaks, frequency of restaurant inspections and food handler training requirements for three consecutive years (2010, 2011 and 2012). Data collection began April 2013.

Results: The preliminary results of this analysis include data from the first 143 completed local health department surveys from 38 states (response rate = 4.9%). In 2012, local jurisdictions varied by number of registered restaurants (mean 946; range 6 to 24,875) and number of restaurant-related foodborne illness outbreaks (mean 1.0; range 0 to 22). The mean restaurant inspection ratio (restaurant inspections per year to registered restaurants) was 2.0 (range 0.7 to 5.0) and restaurant risk classification schemes were inconsistent. The mean restaurant to inspector ratio was 155 (range 12 to 546) and the restaurant inspection to inspector ratio was 291 (range 16 to 1012). One-quarter of local jurisdictions did not require any food handler training, while 17% required a certified food manager on the premises at all times and 29% required all food handlers to obtain a certification card.

Significance: These preliminary results demonstrate heterogeneity of restaurant inspection frequency and food handler training requirements throughout the United States, which may be important factors contributing to restaurant-related foodborne illness outbreaks. To our knowledge, these are the first national data examining an association between restaurant outbreaks and restaurant inspections. Additional data collection is ongoing.

T6-01 Developing Narrative Safe Home Food Preparation Public Service Announcements

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Introduction: CDC estimates that about 20 percent of foodborne illnesses are linked to improper food handling in the home. According to many surveys, most people know the basics of safe home food preparation (Clean, Separate, Cook and Chill), but many people don't comply with safe food preparation practices or follow them inconsistently.

Purpose: The purpose of this project was to investigate barriers to compliance with safe home food preparation practices and to create and distribute narrative public service announcements (PSAs) aimed at encouraging safe food preparation practices by 18-34-year olds who cook regularly for their family and others.

Methods: Formative and summative evaluation was used to design and develop scripts and then create and assess several 30-second narrative television PSAs to improve compliance with recommended safe food preparation practices. Preliminary versions of four 30-second television PSAs were produced and tested in New York, Indiana and Missouri using psychophysiological measures including facial EMG, Skin Conductance, and Heart Rate to determine effectiveness in transmitting the target messages.

Results: Extensive evaluation resulted in four highly and more or less equally effective videos that engaged patterns of cognitive and emotional processing likely effectively communicate about safe food preparation and the risk of foodborne illness. The psychophysiological measures indicated that the selected messages effectively engaged attention. Messages that were explicit about the negative consequences of foodborne illness evoked strong emotional responses that contributed to effectiveness.

Significance: Narrative messages may overcome resistance, address non-compliance, and translate knowledge into behavior by modeling safe behaviors. The use of both traditional and state of the art evaluation to develop food safety PSAs results in effective messages that communicate important home food safety messages.

T6-02 Risk Communication When Ordering Undercooked Hamburgers at Restaurants

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Developing Scientist Competition

Introduction: Ground beef has been associated with *Escherichia coli* O157:H7 and other STECs. As a mitigation step, as cook time of burgers increases, log reduction of harmful bacteria that may be present in ground beef decreases. The U.S. FDA 2009 Food Code states that it is the duty of the restaurant to disclose and remind consumers of risk when ordering undercooked meat products such as ground beef.

Purpose: The purpose of this study was to determine whether the process of disclosure and reminder is actually occurring in chain and independent restaurants that serve hamburgers and to describe it.

Methods: Secret shoppers were recruited and trained on the protocol of hamburger ordering and asking questions about burger doneness and safety. Secret shoppers visited restaurants in several geographic locations throughout the United States, ordered medium rare burgers, and collected risk information on server responses to questions relating to measuring doneness and safety (n = 139). Risk information provided to patrons on restaurant menus was also collected. Codes were developed to characterize server responses based on methods of doneness, and to classify whether safety information and incorrect information were provided.

Results: When secret shoppers engaged with the target population, 81% of servers overall referred to an unreliable method of determining doneness. Fifty-one percent of chain restaurant servers and forty percent of independent restaurant servers provided some sort of safety information; however, 57% of independent restaurant servers and 60% of chain restaurant servers provided incorrect information.

Significance: The majority of servers indicated an unreliable method of doneness or other incorrect information related to burger doneness and safety; these results indicate major gaps in server risk communication. These gaps suggest the need for a food safety curriculum specifically aimed towards servers, with the ultimate goal of improving risk messages to consumers.

T6-03 A Content Analysis of Food Safety Practices in YouTube Beef Hamburger How-to Videos

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Introduction: Ground beef is linked to 41% of *Escherichia coli* O157:H7 outbreaks, and hamburgers account for 68% of these outbreaks. Over 200,000 videos were returned for the term "hamburger" on YouTube, a video sharing community that hosts user-generated video. Little is known regarding food safety practices displayed by content creators.

Purpose: It was hypothesized that YouTube videos display an unfavorable ratio of negative to positive food safety behaviors and home cooks will present a greater ratio than professional cooks or chefs. Evaluating food handling on YouTube is important because modeled practices may have negative effects on consumer food safety behavior.

Methods: Content analysis was used to generate objective data from media to compare concepts, actions, instructions, and other information. Inclusion criteria were developed to select videos with similar number of views, length, and content specific to hamburgers. Coding schedule development was designed to record risk factors present, as well as modeling of positive food safety practices. Coding schedule was developed to include relevant food safety behaviors within the short format common to YouTube.

Results: Negative food safety behaviors were modeled in 100% of sample (n = 89) videos included for analysis. Videos modeled an average of 1.88 negative food safety behaviors, with cross-contamination present in 64 (72%) of the videos. Only 1 (1.1%) video modeled proper use of a food thermometer, with 103 instances of incorrect determination of doneness, and 11 (12.4%) videos utilized multiple risky methods. View counts averaged 170,831 with total view count of 15,204,020.

Significance: The Social Cognitive Theory posits learning occurs by modeling, or observing others behavior and the subsequent consequences. YouTube videos encompass verbal instruction and symbolic behavior that may influence food safety behaviors. Absence of positive behaviors and negative food safety behavior modeling creates learning opportunities that foster risky food safety practices.

T6-04 Development of a Messaging Strategy Using Learning Style Preference Intensity Scores

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Developing Scientist Competition

Introduction: Communication researchers at the National Center for Food Protection and Defense (NCFPD) used Sellnow's (2005) Learning Style Quiz and Venette's (2006) Learning Style Calculator to better understand risk messaging. Learning style research in risk and crisis communication currently suggests that messages have the broadest appeal if they focus on three issues: what is happening, why it is important, and what to do. This project shows that the intensity of preferred learning style also affects acceptance of a food-risk warning. Communication strategies should be adapted accordingly to resonate with individual's learning preferences during a food-contamination event.

Purpose: This project sought to determine if including preference intensity affected acceptance of message recommendations.

Methods: Surveys were included in this analysis using participants (n = 899) from four diverse U.S. locations. Local cultural agents served as recruiters in the communities, and individuals were provided access to an online survey. Sellnow's Learning Style Quiz was administered to participants, and Venette's Learning Style Calculator was used to determine preferred style and intensity.

Results: The calculated preferred learning style and intensity scores were compared to established message strategies. Based on the findings, Venette's method for determining learning style preferences both avoided previous limitations noted in standard learning style preference analysis, and also allowed for richer interpretation of message-outcome results. The revised strategy indicates that food-risk messages appeal to the largest portion of the audiences when they:

1. Tell the story of the crisis (report events),
2. Demonstrate that the event is physically or psychologically close to the audience (propinquity), and
3. Provide reasonable actions with relevant examples.

Significance: Communicators must deliver messages that have the broadest appeal to various publics. Findings indicate that learning style intensity is also important to messaging, and thus a new messaging strategy is presented.

T6-05 Impact of Multi-year Hand-hygiene Training on Florida Citrus Packers' Self-reported, Attitudes, Awareness, and Practices Away from the Workplace

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Developing Scientist Competition

Introduction: Social Cognitive Theory identifies reciprocal determinism; a person can be both an agent for and a responder to change, as a key construct. Transition of employee hand-hygiene training, knowledge, attitudes and behavior skill to hand-hygiene practices away from the workplace for packinghouse workers is lacking.

Purpose: Our purpose was to evaluate relationships between repeated multi-year hand-hygiene training delivered to Florida fresh citrus packers, as part of a larger Citrus Packinghouse Worker Training Program, and their self-reported hand-hygiene attitudes, awareness and practices away from the workplace that may contribute to overall worker health and health behaviors within the community.

Methods: A 45-min, interactive, personal hygiene module, based on Cornell GAP and GlobalGAP curricula, has been offered annually since 2008, resulting in implemented, supported and reinforced hand-hygiene programs. To evaluate the impact of multi-year trainings on self-reported hand-hygiene away from the workplace, a quantitative survey was designed and administered to volunteer English-speaking participants from five packinghouses (n = 74). A separate variable was created for questions relating to three domains (attitudes; awareness, and self-reported practices), giving three composite scores. Overall hand-hygiene score (OHHS) was created by summing responses to 27 weighted questions from composite scores. Frequencies, means, and standard deviations were calculated. Relationships between the domains were assessed with a two-tailed Pearson's correlation; $P \leq 0.05$ was considered significant.

Results: Among packers with >2 trainings, there are significant relationships between the domains. All domains co-vary together, with the strongest relationship (r=0.432; $P < 0.01$) occurring between awareness and self-reported practices. No notable differences in mean responses between genders were seen in the overall scores for each subcategory; participants OHSS were 92.3%, very high given the potential range of scores.

Significance: Florida fresh citrus packers receiving multi-year hand-hygiene trainings in the workplace have high awareness and self-reported practices regarding hand-hygiene in non-work environments.

T6-06 Overcoming Outreach Challenges and Improving Produce Safety Educational Opportunities for Fresh Fruit and Vegetable Farmers

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Introduction: The Produce Safety Alliance (PSA) has developed a science-based, on-farm food safety curriculum for fresh fruit and vegetable farmers to meet the training requirement (§ 112.22(c)) included in the proposed Produce Safety Rule as part of the Food Safety Modernization Act (FSMA).

Purpose: The purpose is to provide a standardized curriculum, training format, and outreach strategy to help farmers prepare to meet the first ever fresh produce regulation. There are approximately 189,000 fresh produce farms in the United States. While not all of these farms will be subject to the regulation, they all provide fresh produce for consumption and therefore impact public health.

Methods: Curriculum learning objectives and content were developed through data collected from eight nationwide farmer focus groups (2013) and the PSA Working Committee process (2011-13) which included a total of 72 meetings involving 178 members comprised of farmers, extension educators, researchers, and produce industry personnel. The PSA Educators Group, currently representing 44 states, has identified challenges and proposed solutions to reaching farmers across the country.

Results: A seven hour curriculum designed to be delivered in one day to farmers and regulators has been developed. Critical challenges in education and outreach have been identified including lack of funding to provide training for farmers, geographical accessibility to farmers, and availability of qualified educators to conduct trainings. Solutions including the PSA train-the-trainer programs throughout the U.S. to build a cadre of qualified trainers and additional requests for funding to supplement outreach efforts to address these challenges are currently being put into action.

Significance: Understanding and overcoming barriers to delivering food safety training will increase farmers' ability to meet regulatory and market requirements for food safety and help prevent fresh produce contamination, in turn protecting the health of those who consume fresh produce.

T6-07 Prevalence and Antimicrobial Resistance Patterns of Major Foodborne Bacterial Pathogens in Mixed Crop-animal Farms and Its Products in Retail Stores and Farmers Markets

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Introduction: *Salmonella*, *Campylobacter jejuni* and enterohemorrhagic *Escherichia coli* (EHEC) are the major causative bacterial agents of foodborne illness. Increasing numbers of outbreaks with these bacterial pathogens have been attributed to vegetables specifically organic products. Mixed crop-animal/back yard farms, possessing higher biosecurity and biosafety risks, are one of the major producers of organic products, supplying farmers market as well as organic stores. It is essential to understand possible ecological sources of contamination at the production level and the translation of contamination at the consumption level.

Purpose: To investigate the prevalence and antimicrobial susceptibility of *Salmonella*, *C. jejuni* and EHEC in pre-harvest and post-harvest products of mixed crop-animal farms.

Methods: Samples (>350) were collected from organic farms, farmers markets, organic and conventional supermarkets located either in Washington D.C. or Maryland. Portions of each sample were diluted 1:9 with buffered peptone water. After enrichment in broth containing 5% sheep blood, samples were streaked on agar; presumptive colonies were confirmed biochemically and PCR analysis. Antimicrobial susceptibility testing was performed using the agar dilution method. The data was analyzed using the χ^2 test.

Results: On organic farms surveyed, there was a significant (P = 0.01) difference in the occurrence of *Salmonella* and *Campylobacter* between environmental, animal feces, animal feed and vegetable samples, with prevalence of 35%, 70%, 22% and 20% for *Salmonella* and 15%, 36%, 12% and 16%, respectively. These numbers are significantly higher than conventional farms. There was no significant difference in the rates of antimicrobial resistance of *Salmonella* and *C. jejuni* recovered from those environments as well as organic and conventional products in markets and farms.

Significance: The study shows contamination at the farm level is significantly higher in mixed farms. These data also suggest that there is no difference in microbiological quality of organic products in markets compared to conventional products.

T6-08 Results of a Photonovella Educational Food Safety Intervention for African Americans of Low Socioeconomic Status

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Introduction: Foodborne illness disproportionally affects the African-American community due to a large percentage of this population living below the poverty level and holiday food preferences that may include pork chitterlings.

Purpose: To determine the efficacy of a photonovella designed to educate and influence behavior related to meat and poultry food safety.

Methods: An educational photonovella was designed after performing focus groups with African Americans of low socioeconomic status. A food safety questionnaire was performed pre and post intervention. A 16-point weighted food safety score was calculated out of 12 questions. Comparisons of specific food safety issues were made to determine efficacy.

Results: Among 149 African American consumers interviewed, the overall mean baseline food safety score rose from 11.4 (71%) to 12.7 (79%) ($P < 0.01$) after reading the photonovella. The proportion of all consumers who said it was okay to thaw ground meat on the counter declined from 27% to 9% ($P < 0.01$). The proportion who knew how long to wash their hands increased from 58% to 69% ($P = 0.01$). Ninety-five (64%) reported making at least one change to their behavior since receiving the photonovella. Thirty-five percent and 16% of the consumers, respectively, said that someone else they lived with and someone else who doesn't live with them also read it. Eighty-two percent of the consumers said they had not previously heard a chitterlings-related food safety educational message. Among 27 consumers who cooked or prepared chitterlings but did not follow the USDA recommendation to boil for 5 minutes before cleaning (preintervention), 10 (37%) reported that they changed their behavior - they now either boil (7) or do not cook (3) chitterlings ($P < 0.01$).

Significance: These data demonstrate that a photonovella on food safety may have a beneficial effect in African Americans of low socioeconomic status and even attract the interest of persons who encountered it outside the study.

T6-09 Food Safety Education Capacity Building Programs in Afghanistan

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Introduction: After 35 years of nearly continuous war in Afghanistan, there is a significant deficit in basic food safety knowledge among many fractions of the Afghan population including the Afghan Ministry of Agriculture, Irrigation and Livestock (MAIL) and the Ministry of Public Health (MoPH) regulatory agencies. Few food producers successfully export their products to high value markets, partially due to the inability to meet basic food safety standards.

Purpose: The purpose of these training programs was to develop basic food safety knowledge and skills in MAIL, MoPH, and select industry personnel to begin the process of increasing food safety and export potential in Afghanistan.

Methods: A food safety education needs assessment was conducted in Kabul, Afghanistan in February 2012. Food safety trainings tailored to current conditions in Afghanistan were developed. Two trainings conducted in Kabul focused on (i) introductory food safety (Good Agriculture Practices, Good Manufacturing Practices, and basic HACCP), and (ii) basic sanitation for MAIL, MoPH, and industry personnel. An intensive 3-week basic food microbiology laboratory training program was conducted in Ankara, Turkey for 10 MAIL and MoPH government officers. Pre-and post-training assessments were conducted for all training programs.

Results: A total of 27, 38, and 10 MAIL, MoPH, or industry personnel were trained in the Introductory Food Safety, Basic Sanitation, and Basic food microbiology trainings, respectively. In each training there was a statistically significant increase in understanding of the training materials (paired *t*-test; $P < 0.05$). Each cohort indicated need for (i) continued training in these areas to improve their ability to train others and (ii) increased laboratory capacity.

Significance: These training programs are part of foundational effort to rebuild knowledge and regulatory capacity in the Afghan MAIL and MoPH, which will ultimately lead to increased food security through enhanced food safety.

T6-10 Withdrawn

T6-11 Estimates for the Cost of Foodborne Illness across U.S. States

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Introduction: Understanding the costs associated with foodborne illnesses is important to policymakers both as a way to prioritize resources for harm mitigation and as a tool in assessing whether proposed interventions improve social welfare. At the national level, multiple methods of assessing costs have been used by Federal food safety regulatory agencies in regulatory impact analyses. The use of national estimates by state policymakers may result in incorrect assessments of costs, possibly leading to inefficient resource allocation.

Purpose: The purpose of this study was to use three methods of measuring foodborne illness costs to generate state-level costs.

Methods: Federal estimates for the number and severity of illnesses for each pathogen in each state are used. Three methods are used to assess costs. The most conservative of these methods uses only medical costs and productivity losses to estimate costs. The most comprehensive method uses adjusted value of statistical life (VSL) estimates to approximate non-financial utility losses associated with illness and death. A third model uses VSL estimates for deaths, but not for nonfatal illnesses. Costs for medical care and productivity losses are based on publically available state-specific medical cost and compensation cost data. Costs for the VSL are based on a meta-analysis, adjusted for differences in state income.

Results: Numerous estimates were derived. For example, medical costs for an average case of foodborne illness range from a low of $90 in West Virginia to a high of $150 in New Jersey, while medical costs associated with *Vibrio vulnificus* are much higher, ranging from $25,700 in Maryland to $62,000 in New Jersey. Similar estimates were derived for each pathogen and cost category.

Significance: Foodborne illness cost estimates vary significantly by state and method, suggesting careful use of the most relevant estimates.

T6-12 Strengthening Food and Water Safety in Canada through an Integrated Federal Genomics Initiative

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Introduction: Health Canada is leading a team of 53 scientists from across Canada representing 6 federal government Departments/Agencies (Health Canada, Canadian Food Inspection Agency, Agriculture and Agri-foods Canada, Public Health Agency of Canada, National Research Council of

Canada, Environment Canada) on a major collaborative Pilot project under the umbrella of the Genomic Research and Development Initiative (GRDI) program.

Purpose: The Food and Water Safety (FWS) project targets two priority food and water pathogens; *E. coli* (O157 and non-O157) and *Salmonella* Enteritidis. The objectives are: 1) develop rapid methodologies and tools for detection and source track these pathogens from farm to consumer, 2) provide genomic tools for risk assessment, epidemiological analysis, and intervention strategy development, 3) create of a national genomic database, and 4) supporting the development of needed guidelines and policies to enhance food and water safety.

Methods: The FWS has 3 major themes; 1) *Isolation and Detection* to rapidly detect and identify these pathogens in foods and water, 2) *Information Generation* through the development of comprehensive national genomic database of whole genome sequences of strains as well as source attribution, and 3) *Bioinformatic* analysis.

Results: "Priority six" non-O157 VTEC (O26, O45, O103, O111, O121 and O145), reference strains have been selected. Completed development of rapid isolation methods, functional PWEF sensor microarrays for *E. coli*, achieved rapid enrichment of VTEC from 1 cell per 65 g in 3-5 hours, and developed a functional prototype integrated Lab-on-A-Chipmicrofluidics-based for rapid capture of whole VTEC cells from food matrices and subsequent identification and characterization. Methods developed aim at reducing time of bacterial isolation, detection and confirmation from days to only few hours. Whole genome sequencing and bioinformatics work is ongoing where more than 200 strains have already been sequenced.

Significance: Outcome should contribute to improving food and water safety and would support development of needed policies and guidelines.

T7-01 On-farm Food Safety Decision Trees: Helping Farmers Assess Risks, Prioritize Practices and Use Resources Effectively

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Introduction: Food safety is every fruit and vegetable grower's responsibility; however, identifying and prioritizing food safety risks on the farm is often difficult. While there are many food safety resources and plan templates offering guidance on Good Agricultural Practices (GAPs) to reduce risks, most do not explain how to assess risks or how to prioritize which food safety practices should be put in place first.

Purpose: The purpose of this project was to develop on-farm food safety decision trees to help growers identify food safety risks and prioritize the effective implementation of practices with limited human and financial resources.

Methods: An advisory group of growers and regulatory personnel worked with researchers to design a decision tree model that was easy to understand and provided the necessary resources to facilitate risk assessment and GAPs implementation with an emphasis on small farms. Scientific recommendations within the decision tree were developed using data from refereed journal articles, industry guidance documents, and expert opinion when research data was unavailable.

Results: A complete On-Farm Food Safety Decision Tree portfolio has been developed including an introduction on how to use the decision tree, a checklist for prioritizing the implementation of practices, a glossary, and nine topic specific area trees including Worker Health, Hygiene and Training; Agricultural Water for Production; Soil Amendments; Wildlife and Animal Management; Land Use; Postharvest Water; Sanitation and Postharvest Handling; Traceability; and Transportation. Each tree can be used independently so growers can adopt practices at their own pace and tailor their food safety plans to their individual farms.

Significance: Providing guidance on how to assess on-farm food safety risks will help growers target the use of limited farm resources to most effectively reduce contamination risks to fresh produce. Preventing contamination will reduce produce-associated foodborne illness outbreaks and protect fresh produce consumers.

T7-02 Thermal Inactivation of Hepatitis A Virus in Spinach

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Developing Scientist Competition

Introduction: Leafy vegetables have been recognized as important vehicles for foodborne viral transmission. To control hepatitis A virus (HAV) outbreaks associated with mildly heated (e.g., blanched) leafy vegetables, generation of adequate thermal processes is important for both consumers and the food industry.

Purpose: The purpose of this study was to (i) characterize the thermal inactivation behavior of HAV in spinach, (ii) compare first-order and Weibull models in describing the data in terms of selected statistical parameters, and (iii) calculate z-values and activation energy using each model.

Methods: Commercial frozen chopped spinach (25-g samples) were homogenized and inoculated with 5-ml of HAV stock (7.20 ± 0.79 log PFU/ml) and held at 4°C for 24h. Inoculated samples (6 ml) were individually vacuum sealed in plastic bags to -100 kPa and heated in a thermostatically controlled water-bath with monitored temperature. Survivor curves (D-values) and thermal death curves (z-values) were generated using different treatment times (0-6 min) at 5 different temperatures (50 to 72°C) by Weibull and first-order models. A comparison test (ANOVA) was used to analyze the effects of temperature and time on survival ratios by the two models.

Results: D-values for HAV in spinach ranged from 37.08 ± 3.37 to 0.93 ± 0.09 min for Weibull and 34.40 ± 4.08 to 0.91 ± 012 min for the first-order model at 50 to 72°C, respectively. z-values for HAV in spinach were 15.07 ± 1.63°C using the Weibull model and 13.92 ± 0.87°C using the first-order model. Calculated activation energies for the first-order and Weibull models were 162 and 151 kJ/mole, respectively. Using this data obtained and the thermal parameters of industrial blanching for spinach as a basis (100°C for 120-180s), blanching of spinach in water at 100°C for 120-180s under atmospheric conditions will provide > 6 log reduction of HAV using either model.

Significance: These results will benefit the frozen food industry in designing blanching conditions to inactivate/control HAV in spinach.

T7-03 Analysis of Chlorine Replenishment Process of Lettuce Washing Water and the Evaluation of Several Strategies for Improving Water Quality

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Introduction: Sanitizer concentration and efficacy on pathogen inactivation are significantly reduced by organic matters in produce wash water. However, the understanding of fresh-cut produce wash water chemistry, including the organic constituents present, and its potential for depleting chlorine is lacking.

Purpose: We identified major chemical compounds and functional groups in various leafy green vegetable latex, investigated their effects on sanitizer degradation, and explored new approaches for reducing organic load and maintaining sanitizer efficacy.

Methods: Wash waters collected during fresh-cut produce processing were treated with chlorine solutions containing a range of free chlorine concentrations. The treatment solutions were examined with Raman and infrared spectroscopy, and/or subjected to chemical analysis. Several flocculants over a range of concentrations followed by filtration through activated carbon and ozone were used to treat the simulated produce wash water. Turbidity, pH, chemical oxidation demand (COD), total soluble solids, total suspended solids, and chlorine demand were then determined.

Results: Chlorine concentrations influence the transformation of different functional groups in the wash water as shown by Raman and infrared spectroscopy. High molecular weight flocculent significantly reduced wash water turbidity. Flocculation followed by filtration further reduced turbidities from 50 to <1 NTU, but with limited reduction in COD. With activated carbon filtration, COD was reduced 45% from initial 1270 mg l-1 to 700 mg l-1.

Significance: This study provides critical information on the chemistry of produce wash water during chlorine replenishment. Research findings can be used by the produce industry to develop strategies for maintaining sanitizer concentration required to prevent pathogen survival and cross-contamination during commercial produce washing operations.

T7-04 Development and Application of Enhanced *Listeria* Detection and Recovery from Leafy Green Crop Residue: Soil Matrix

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Introduction: Accurate determination of environmental prevalence and on-farm sources of *Listeria monocytogenes* for fresh produce is essential for risk determinations.

Purpose: Accurate determination of environmental prevalence and on-farm sources of *Listeria monocytogenes* for fresh produce is essential for risk determinations.

Methods: Different base-nutrient concentrations of enrichment media were compared for detection of *Listeria* sp. and Lm after 48h incubation at 35°C. The sample matrix was leafy green residues incorporated into diverse soils. Detection by agar culture, conventional PCR, qPCR, and commercial kit PCR were assessed. Field plots of red chard were inoculated with log 7 CFU/l of rifampicin resistant *L. innocua* (TVS451) and soil-incorporated after 18h. Soil was sampled over 77 days and processed using the various methods. In commercial fields, soil was collected from 195 locations following incorporation of a leafy green crop. Fifteen 500g samples were collected 7-10 days after incorporation or following the first replant irrigation at the depth of residual moisture.

Results: Post-enrichment detection of Lm by PCR was greatly enhanced 2X Listeria Enrichment Broth with supplements and 1) detection using the Roka Atlas system for primary PCR; 2) plating on R&F LisLm agar; and 3) species and colony confirmation using probe-based qPCR. Red chard: clay loam soil samples were positive for TVS451 for at least 77 days depending on specific methods used for enrichment and detection. Application of the optimized protocol to diverse commercial leafy greens soils did not result in positive detection. One creek sediment sample was positive for *Listeria* spp.

Significance: Perceptions of the 'ubiquitous' prevalence of Lm in produce production environments can alter the prioritization of preventive controls validation and implementation. Although preliminary, this study indicates that focus on postharvest handling and processing environments may be a higher priority.

T7-05 Transfer and Survival of STEC to Cantaloupe from Surface Irrigation Water

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Introduction: Water used for irrigation is one of the most likely points of pathogen contamination during fruit and vegetable production. While irrigation water is a known point of contamination, there are very few studies that can be used to determine pathogen transfer from contaminated irrigation water to produce and the correlation of water indicator organisms (generic *Escherichia coli*) with pathogen concentration.

Purpose: Evaluate the transfer of Shiga toxigenic *E. coli* (STEC) from contaminated surface water to cantaloupe.

Methods: Cantaloupe plots containing cross-classified combinations of overhead or surface drip irrigation along with bare ground or plasticulture raised bed preparation were irrigated from a pond naturally harboring STEC. Surface water was sampled weekly for enumeration of STEC, generic *E. coli*, and coliforms using routine enumeration methods. Cantaloupes were harvested, enriched in mTSB with 8 mg/l sodium novobiocin, DNA extracted and tested for the presence/absence of *stx* and *eae* genes using multiplex PCR.

Results: Over six weeks, STEC populations in irrigation were found to fluctuate between 0.7 to 2.68 log CFU/100 ml. There was no significant correlation between populations of STEC and coliforms ($r^2 = 0.56$; $P < 0.25$) in irrigation water. A significant correlation was not observed between generic *E. coli* and STEC ($r^2 = 0.41$; $P < 0.45$). Over a four-week harvest period, 210 cantaloupes were sampled for STEC contamination. Bare ground plots with drip irrigation and plasticulture plots with overhead irrigation had the highest occurrence of STEC contamination at 20.4% and 19.7%, respectively. Lower percentages of positive samples were found on overhead irrigated bare ground plots (14%) and drip irrigated plasticulture plots (12%).

Significance: These data suggest that the population of generic *E. coli* in irrigation water does not correlate with STEC concentration. Regardless of irrigation method and bed preparation, when high levels of STEC persist in irrigation water, transfer to cantaloupe can occur.

T7-06 The FACET Software: Databases and Models to Assess Dietary Exposure to Food Additives in Europe

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Introduction: Estimating the dietary dose to food additives in a population requires a number of inputs. The concentration of additive in food must be known, together with the level of consumption of the food. In order to assess total aggregate dose to an additive, this must be known for all foods in the diet, together with the variability in the dietary intake of the foods in the population of interest.

Purpose: To develop databases, models and software to estimate consumer exposure to food additives.

Methods: 15 dietary surveys from 8 member states were recoded into a harmonised food categorisation system for food additives. Market wide data on the distribution of food additive use in the EU was recoded into the same harmonised system, in collaboration with the membership of FoodDrinkEurope. Statistical distributions were constructed from ranges of food additive use, to represent the distribution of use and to overcome

confidentiality issues with the data being linked to a specific food manufacturer. This is turn was linked to a probabilistic dietary exposure model based on food consumption across Europe.

Results: The developed databases and models were integrated into a desktop software system. Options in an exposure assessment include assessing specific food types, using industry data or regulatory limits (Maximum Permitted Levels), and specific consumer demographics. A distribution of exposure per unit body weight is generated. Exposure in the population can be broken down by food category to examine what food categories are driving the exposure.

Significance: The software tool presents a rational and realistic methodology for assessing dietary exposure to food additives in Europe. This enables both industry and regulators to assess whether current additive use levels are protective for human health, and to prospectively assess the impact of changing additive concentrations and Maximum Permitted Levels.

T7-07 Problem of Fish Fermentation at High Temperature for "Guedj" Production in Senegal and Potential by Bacteriocinogenic Lactic Acid Bacteria Strains Used as Starter Cultures on a Local Cereal-based Liquid Matrix to Control Spoilage Bacteria

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Introduction: This study describes the conditions of fermentation in Senegal for production of fish guedj which is a popular condiment and animal protein source across the Sahel, and the capacity by two nisinogenic *Lactococcus lactis* subsp. *lactis* strains, used as starter cultures.

Purpose: To improve the control of spoilage bacteria.

Methods: Lactococcal strains have been qualitatively determined as suitable hurdle for use on fish within four bacteriocinogenic lactic acid bacteria, on the basis of their negative histidine and tyrosine decarboxylase activity. They growth well on a new-developed liquid matrix based on millet flour, a local cereal.

Results: Fish are transformed to guedj by spontaneous fermentation for 24 to 48h at ambient temperatures near 30°C followed by salting (with NaCl) and sun drying. Spontaneous fermentation of fish generally performed by its immersion in salted sea water led to the proliferation of enteric bacteria to 9 log CFU/g.

By immersion of two lean (*Podamassys jubelini*) and fat (*Arius heudelottii*) fish from artisanal production in the newly formulated substrate inoculated with 10^{6-7} CFU/g of the pure starter cultures, the pH was reduced and maintained below 4.5 for 48 h at 30°C sufficient to inhibit pathogenic and spoilage bacteria. When the fish starter culture fermentation is carried out in presence of a nisin sensitive pathogenic *Listeria monocytogenes* strain inoculated at 10^5 CFU/g, the level of this indicator was reduced below 10^2 CFU/g while in control samples (fermented without LAB living culture addition), it reached 10^8 CFU/g.

Significance: These results indicate that the new fish fermentation approach by treatment with the two nisin producers could enable improvement of guedj safety.

T7-08 Native Microbial Populations of WPC34 and WPC80 Whey Protein: Effect of Storage Temperatures on Survival and Growth

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Introduction: Whey proteins (WPC34 and 80) have been used as food ingredients and as bases for making biodegradable products. However, there is limited information on the behavior of native microflora associated with these products.

Purpose: The objective of this study was to estimate classes of native microflora of WPC34 and WPC80 using different agar media and then investigate how storage temperatures (5, 10, 15, 22 and 30°C) for 7 days would affect survival and growth of each class of the bacteria estimated.

Methods: Immediately after receiving WPC34 and WPC80 from the manufacturer, the initial populations of aerobic mesophilic bacteria, coliform, yeast and mold, lactic acid bacteria including lipolytic bacteria were enumerated, and bioluminescent adenosine triphosphate (ATP) assay was used to estimate ATP level corresponding to the overall microbial populations associated with WPC34 and WPC80 products.

Results: Total microbial populations in WPC34 and WPC80 determined immediately and after storage for 7 days averaged 6.8 log and 7.1 log CFU/g, respectively, and the corresponding ATP values associated with the total microbial populations averaged 62 and 73 RLU, respectively. Class of microorganism estimated from WPC80 averaged 2.8 log CFU/g for aerobic mesophilic bacteria, below detection (<2 cfu="" g="" for="" yeast="" and="" mold="" coliform="" bacteria="" as="" well264="" log="" lipolytic="" lactobacillus="" respectively="" wpc34="" aerobic="" mesophilic="" determined="" averaged="" 3015="" below="" detection="" storage="" temperatures="" did="" not="" cause="" significant="" $P > 0.05$) changes in total microbial populations of WPC34 and WPC80.

Significance: The results of this study suggest that storage temperature up to 30°C for 7 days would not change the native microbial population and that the bioluminescent ATP assay can effectively be used to estimate total microbial populations in WPC34 and WPC80 products.

T7-09 Effect of Alkaline pH on the Heat Resistance of *Salmonella* Enteritidis

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Introduction: Egg white is widely used in food formulation for its emulsifying properties; however, this ingredient may be contaminated by *Salmonella* Enteritidis. To ensure food safety, heat treatment should be applied with a temperature no more than 62°C. Moreover egg white also presents the particularity to have an alkaline pH close to 9.2 which could be taken into account to optimize pasteurization process.

Purpose: The aim of this study was to quantify the effect of alkaline pH on the heat resistance and the revivification of *Salmonella* Enteritidis.

Methods: Thermal inactivation kinetics of *Salmonella* Enteritidis NCTC 13349 were performed using capillary method. Different conditions for treatment and incubation media were studied from pH 7 to 10. Inactivation kinetics were fitted using the Weibull model and the effects of the heating and recovery pHs on the bacterial resistance were quantified using a modular model approach.

Results: Concave shaped inactivation kinetics (log CFU vs heating time) were fitted by the Weibull model in which parameter "δ" (first decimal reduction time) quantifies bacterial heat resistance. A large effect of alkaline pH of treatment and recovery media was observed on heat resistance. An increase of treatment medium from pH7 to pH9.5 leads to a fivefold reduction the *Salmonella* Enteritidis NCTC 13349 heat resistance. Whereas an increase from pH 7 to pH 9.5 of the recovery medium leads to a two-fold reduction of heat treatment time for the same efficiency. Thus, heat treatment at 56°C for both treatment and incubation media at pH7 presents similar bacterial reduction than a heat treatment at 52.3°C at pH 9.2.

Significance: Taking into account the alkaline pH of egg white during pasteurization and storage allows minimizing heat treatment intensity. These results ensure a risk/benefit approach yielding destruction of *Salmonella* Enteritidis and functional properties of egg white.

T7-10 Rapid Response to an Outbreak of *Listeria monocytogenes* Infections Associated with Brand A Soft Cheese: Early Collaboration between the FDA, CDC and State Partners

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Introduction: *Listeria monocytogenes* (LM) causes severe illness and death in susceptible individuals, including newborn infants, older adults, and immunocompromised patients, in addition to fetal infections in pregnant women. LM is acquired through consumption of contaminated food.

Purpose: In late June and July 2013, CDC, FDA's Coordinated Outbreak Response and Evaluation Network (CORE) and state partners investigated an outbreak of five LM cases from four states, including one adult death and one fetal loss; the isolates had indistinguishable PFGE patterns. Rapid epidemiologic, traceback, and molecular analyses were used to identify the contaminated food and prevent additional illnesses.

Methods: State and local partners shared epidemiological information with CDC. CDC notified CORE of the cluster and identified soft cheese epidemiologically as a suspect exposure. FDA/CORE's research of past environmental isolates matching this PFGE pattern combination identified a Wisconsin cheese manufacturer (Company A) with a history of colonization by this LM strain. CORE shared this information with CDC to assist the epidemiologic investigation. State and local partners collected and analyzed household and retail products, in addition to environmental samples. FDA/CORE conducted a traceback investigation from several points of service to Company A, while FDA and the Wisconsin Department of Agriculture jointly inspected Company A.

Results: All patients reported definite or probable consumption of a Company A cheese. The LM outbreak strain was isolated from two retail samples collected by Minnesota Department of Agriculture and one product sample collected during inspection of Company A. The traceback and facility investigations determined the contamination most likely occurred at Company A. The firm ceased all cheese production and voluntarily recalled its product. Root cause analysis efforts are ongoing.

Significance: This outbreak highlights the effectiveness of federal and state collaboration to rapidly identify and remove a contaminated product from the marketplace, thereby protecting public health.

T7-11 Application of Food Defense Software Tools for the Purposes of Informing Intervention Strategies

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Introduction: Intentional foodborne contaminations have been attempted in the United States and abroad. As part of Terrorism Risk Assessment efforts for the Department of Homeland Security, software tools capable of assessing various threats to the U.S. food supply from 'farm-to-fork' have been developed. Such, quantitative software tools for food defense can be used to prioritize strategic initiatives (e.g., investments in diagnostics and assays), as well as operational activities (e.g., epidemiological investigations), in a manner that quantifies benefits for decision makers.

Purpose: The purpose of this study was to identify opportunities for strategic investments and initiatives to reduce risk by better understanding the potential impact of key aspects, specifically the speed of intervention strategies (e.g., a recall, advisory, or warning) and the compliance of both consumers and retailers with interventions.

Methods: Through collaboration with the National Center for Food Protection and Defense (NCFPD) and the Department of Homeland Security Office of Health Affairs (OHA), the universe of food scenarios was condensed to combinations of representative food clusters and toxidromes (i.e., a group of chemicals resulting in similar human health effects and requiring similar medical treatments). Quantitative mathematical models were then constructed for exemplar foods within each cluster based on an extensive data gathering effort involving the United States Department of Agriculture (USDA), the United States Food and Drug Administration (FDA), trade associations, and food industry. Model results were generated for a representative chemical within each toxidrome to reflect the food cluster-toxidrome combination. Simulations for each cluster-toxidrome combination were performed using current baseline capabilities, as well as alternate conditions to assess the impact on consequences of faster investigation timelines (e.g., the benefit of advanced, rapid diagnostics) and enhanced intervention compliance.

Results: Comparison of results for simulations indicate key areas where improved diagnostics, early warning systems based on public health data, public education programs, and the use of social media to spread information can significantly impact the potential human health consequence of foodborne contamination scenarios.

Significance: This presentation provides a clear example of how quantitative software tools, developed for the Department of Homeland Security, can fuel risk mitigation strategies to better protect the US homeland.

T7-12 Population Dynamics of Generic *Escherichia coli* and Surrogate *Escherichia coli* O157:H7 in Manure-amended Soils

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Introduction: Proposed U.S. FDA standards for untreated biological soil amendments stipulate a 9-month waiting period between soil application and harvest to reduce the risk of pathogen contamination on fresh produce. Manure, soil type, and initial bacterial populations may impact survival of bacterial pathogens and risk of produce contamination.

Purpose: To compare population dynamics of non-pathogenic *Escherichia coli* (*gEc*) and attenuated *E. coli* O157:H7 (*attEc*) strains in surface and 15-cm cores of soils amended with untreated animal manure.

Methods: A multi-strain inocula of *gEc* and *attEc* containing low (LC) and high (HC) populations ($5.3x10^4$ and $3.8x10^6$ CFU/m^2), respectively, were surface-sprayed onto conventional and organic field plots (2m^2) amended with poultry litter (PL), dairy-manure solids (DS), horse manure (HM), or no manure (NM). Manure was tilled into the soil in half the plots. Survival in surface and 15-cm core (tilled plot) samples was determined over 180 days post-inoculation (dpi) by colony count or MPN procedure.

Results: With few exceptions, LC- and HC-manured soil populations (surface and core) of *gEc* were reduced from 2.7-3.5 log CFU/g and 3.3-5.4 log CFU/g, respectively, to <1.0 log MPN/g by 7-28 dpi. Populations of *gEc* from HC-PL in conventional and organic soils survived up to 120-150 dpi and to 90 dpi in LC-PL before declining to <1.0 log MPN/g, respectively. Populations in HC-HM and HC-DS did not fall to <1.0 log MPN/g until 56 and 90 dpi,

respectively. Populations of *attEc* consistently declined more rapidly than those of *gEc* in the same treatments, and *attEc* never resuscitated to >1.0 log MPN/g as did *gEc*.

Significance: Conventional and organic soil amended with PL consistently prolonged survival of both gEc and *attEc* compared to soils amended with DS, HM, or NM. Population declines for *gEc* generally were moderated in surface compared to core soils, but *attEc* populations were very similar in surface and core soils.

T8-01 Allyl Isothiocyanate Vapor Reduces Populations of Foodborne Pathogens on the Surface of Cantaloupe (*Cucumis melo* L. var. *reticulatus*)

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Introduction: Allyl isothiocyanate (AITC) has been shown to have antimicrobial activity in both liquid and vapor states. Due to its high volatility, AITC vaporizes faster at increased temperatures, and thus may be more successful in reducing foodborne pathogens at higher temperatures.

Purpose: The purpose of this study was to determine if exposure to AITC vapor for different time and temperature combinations reduces *Salmonella enterica* serovar Michigan and *Listeria monocytogenes* on the surface of 'Athena' cantaloupe (*Cucumis melo* L).

Methods: Fifty µl of *S.* Michigan or *L. monocytogenes* was inoculated onto whole cantaloupes in 22mm diameter circles and allowed to dry for 90 min. (final inoculation was 6.35 or 6.30 log CFU/circle, respectively). Each cantaloupe was placed inside a 12l glass desiccator and exposed to AITC vapor or control (no treatment) at 25 or 35°C for 1 or 24h. Vapor was created by dispensing liquid (either 100 or 300µl) onto Whatman No. 1 filter paper and attaching it to the lid of the desiccator prior to sealing. Following treatment, the 22mm diameter sections were removed, homogenized and plated onto appropriate agar.

Results: Treatment of cantaloupe for 24h resulted in lower populations of pathogens than 1h ($P < 0.05$). However, there were no significant differences in pathogen recovery between vapor treatment volumes (100 or 300µl) after 24h at 35°C. The higher vapor concentration at 25°C for 24h caused the greatest reduction of *S.* Michigan (3.92 log CFU/10 g) and at 35°C for 24h against *L. monocytogenes* (3.86 log CFU/10 g). At 35°C the higher vapor concentration caused the greatest reduction of both pathogens after 1h (3.68 log CFU/10 g for *S.* Michigan and 1.75 log CFU/10 g for *L. monocytogenes*, respectively).

Significance: AITC vapor shows promise as a way to reduce pathogens on fresh cantaloupe.

T8-02 Mechanisms of Action of Plant Antimicrobials against Murine Norovirus

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Introduction: Numerous plant products possess antibacterial activities; however, limited research has been performed with viruses.

Purpose: To determine the efficacy and the antiviral mechanisms of action of six plant antimicrobials against murine norovirus type 1 (MNV-1), a human norovirus surrogate.

Methods: MNV-1 was used in tests with oregano oil (OO), carvacrol (CV), lemongrass oil (LO), citral (CI), allspice oil (AO), and clove bud oil (CBO). In addition to a cell infectivity assay, an RNase protection assay, a cell binding assay, and transmission electron microscopy (TEM) were performed to elucidate the antiviral mechanisms of action.

Results: OO, CV, and AO produced significant ($P \leq 0.05$) reductions in MNV-1 of 1.0-1.8-log within 15-30min of exposure. CV was the most effective, resulting in a 3.9-log reduction within 1h. LO, CI, AO, and CBO required 24h to achieve similar reductions (2.7-,3.0-,3.4-,2.9-log, respectively). OO only achieved a 1.1-log reduction after 24h. OO, CV, AO, and CBO had no effect on virus adsorption to host cells; LO and CI caused indiscriminate binding to host cells/culture plates. OO, CV, AO, and CBO all caused a loss of MNV-1 capsid integrity, exposing the genome to the effects of the RNase enzyme. The MNV-1 treated with LO and CI was unaffected. Under TEM, all six antimicrobials caused expansion of the virus particles from ≤35nm to up to double in size (OO, AO, CBO) or larger (500-800nm; LO, CI, CV). With CV and CBO, virus capsid disintegration was directly observed.

Significance: These antimicrobials appear to act upon the non-enveloped MNV-1 through a variety of mechanisms such as the disintegration of the virus capsid, degradation of the RNA genome, and coating of the capsid, thereby preventing specific adsorption to host cells. Understanding the mechanisms of such antiviral activity is important in order to predict the efficacy of similar compounds against foodborne viral pathogens.

T8-03 Understanding Antimicrobial Activity of Chitosan Microparticles against *Escherichia coli* O157:H7

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Introduction: Antibiotics have been fundamental to support human and animal health. However, the essential role of antibiotics has been challenged by the occurrence of antimicrobial resistance (AR). The rise of AR is a tremendous concern, and problems of AR are growing in importance mainly because new antibiotic development is not keeping pace with the emergence of AR. Recently, it has been shown that oral feeding of chitosan microparticles (CMs) reduces *Escherichia coli* O157:H7 in cattle, suggesting potential use of this agent as an alternative antimicrobial agent, but the mode of action was not clearly understood.

Purpose: The purpose of this study was to understand the underlying mechanisms of antimicrobial activity of CMs against *E. coli* O157:H7.

Methods: Antimicrobial assay was conducted using CMs at various concentrations at different pH. Live/dead assay was performed to test if bacterial membranes were disrupted by CMs. Gene deletion was performed to identify CMs binding targets using λ-Red mutagenesis system. *In vitro* CMs binding assay was used to determine the binding activity of CMs to *E. coli* O157:H7.

Results: Binding assays and genetic studies with an *ompA* mutant strain demonstrated that outer membrane protein OmpA of *E. coli* O157:H7 is critical for CMs binding, and this binding activity is coupled with a bactericidal effect of CMs at neutral pH. In addition, we found that LPS is another binding target of CMs that enhances antimicrobial activity at acidic pH. Furthermore, CMs showed remarkable antimicrobial activity against Gram-negative and Gram-positive pathogens such as *E. coli*, *Salmonella*, *Vibrio*, and *Streptococcus*, exerting a broad spectrum of activity.

Significance: These data demonstrate how CMs interacts with pathogens to exert antimicrobial activity and we propose that CMs hold potential use as a natural antibiotic to reduce the risk of foodborne pathogens.

T8-04 *Quillaja saponaria* Extract to Control the Spread of *Escherichia coli* O157:H7 and the Emerging Non-O157 Shiga Toxin-producing *E. coli*

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Developing Scientist Competition

Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) O157:H7 and the emerging big six STECs are important food safety concerns. Natural plant-based alternatives for control are gaining popularity. Aqueous *Quillaja saponaria* bark extract (QE) has U.S. Food and Drug Administration approval as a food additive. QE contains bioactive polyphenols, tannins and saponins, with anti-inflammatory and antimicrobial activity.

Purpose: The objective of this study was to determine the effects of QE against *E. coli* O157:H7 and non-O157 STECs over 16h at 37ºC and RT.

Methods: Overnight cultures of 5 strains of *E. coli* O157:H7 and 6 non-O157 STECs were grown in Tryptic Soy Broth, washed, resuspended in phosphate-buffered saline (PBS, pH 7.2), and treated with QE, citric acid (pH 3.75), sodium benzoate (0.1% w/w) or PBS (control) for 6 or 16h at RT and 37°C. Treatments were serially diluted and plated on Tryptic Soy Agar and enumerated after 24h. Data from duplicate treatments replicated thrice were statistically analyzed (ANOVA). Scanning electron microscopy (SEM) was used to determine bacterial structural changes.

Results: Reductions ranging from 0.71 ± 0.09 to 2.95 ± 0.31 log colony forming units (CFU) were obtained for four *E. coli* O157:H7 strains treated with QE after 6 and 16h at RT. However, O157 strain ATCC 43894 showed at least 4-log reduction after both 6 and 16h, with ~1-log increase at 37°C. For 5 non-O157 STECs, 0.85 ± 0.12 to 1.75 ± 0.02 log reductions were obtained after 6 and 16h, with 1.1 ± 0.33 and 2.95 ± 0.32 log reduction for O145 after 6 and 16h with QE at RT, respectively, and higher reductions at 37°C. Citric acid and sodium benzoate controls showed no reduction. Overall, reductions were strain-dependent. SEM analysis showed structural deformations of treated bacteria.

Significance: QE shows antibacterial effects for potential application in food systems. Further research involves determination of the mechanism of action and evaluation in food systems.

T8-05 Control of Hepatitis A Virus by Blueberry Juice and Blueberry Proanthocyanidins

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Developing Scientist Competition

Introduction: Hepatitis A virus (HAV) is an epidemiologically significant foodborne virus that causes severe disease symptoms, with 1.4 million annual cases reported worldwide. Novel approaches to prevent and control HAV transmission are therefore necessary. Blueberries contain high amounts of flavonoids, including proanthocyanidins (PAC) that have various health benefits and antimicrobial properties.

Purpose: This study aimed to (1) determine the effect of blueberry juice (BJ) and blueberry proanthocyanidins (BB-PAC) on the infectivity of HAV at 37ºC over 24h; (2) understand the mode of action of BB-PAC on viral adsorption and viral replication; and (3) determine viral structural changes after BB-PAC treatment using transmission electron microscopy (TEM).

Methods: HAV at ~ 5 log PFU/ml was mixed with equal volumes of BJ (pH=2.8), neutralized BJ (pH=7), BB-PAC at 1, 2, or 5 mg/ml, phosphate buffered saline (PBS, pH 7.2), or malic acid (pH=3) for up to 24h at 37ºC. Treated viruses were serially diluted and plaque assayed in duplicate using three replications, and recovered titers were statistically analyzed. Pre-infection and post-infection BB-PAC treatments of host cells were carried out to determine effects on viral adsorption or replication, respectively. TEM analysis was used to determine treatment effects on virus structure.

Results: HAV titers were reduced to undetectable levels after 30 min with 2 and 5 mg/ml BB-PAC, to undetectable levels after 3h with 1 mg/ml BB-PAC, and by 2.01 ± 0.08 log PFU/ml after 24h with BJ. BB-PAC at 0.5 mg/ml did not show any effect on HAV replication and adsorption. Malic acid (pH control) did not cause any significant titer reduction after 24h. TEM revealed structural damage of HAV particles.

Significance: BB-PAC is effective in decreasing HAV infectivity. BB-PAC can damage the viral capsid and thus prevent host-cell entry. Further studies that incorporate BB-PAC in food systems are needed for its application to prevent HAV transmission/outbreaks.

T8-06 *In vivo* Evaluation of Antimicrobial Activity of Chitosan Microparticles in Cows with Uterine Disease

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Introduction: Antibiotic drugs have been key elements to sustain human and animal health. However, the essential role of antibiotics has been challenged by the occurrence of antimicrobial resistance (AR). The rise of AR is a tremendous concern for public and animal health and problems of AR are growing mainly because new antibiotic development is not keeping pace with the emergence of AR. Chitosan microparticles (CMs) have been developed as a natural antimicrobial agent, and *in vitro* results provide promising use for disease treatment.

Purpose: The purpose of this study was to evaluate *in vivo* antimicrobial activity of CMs in an animal model, cow uterus with uterine disease.

Methods: For *in vivo* animal experiments, Holstein cows with metritis (n = 30) were randomly assigned to one of three treatments as they were diagnosed with metritis: CMs treatment, Ceftiofur antibiotic treatment, and no treatment. To evaluate the efficacy of CMs, we enumerated intrauterine pathogenic *Escherichia coli* (IUPEC) in uterus. Uterine swab samples were daily collected for 7 days and enumerated using CHROMagar *E. coli*.

Results: CMs treatment effectively reduced the shedding of IUPEC in the cow uterus. The efficacy of antimicrobial activity was better than ceftiofur in the reduction of this pathogen. Cows treated with CMs stopped the shedding of IUPEC within five days of treatment, while 50% of ceftiofur treated cows shed for more than seven days. In addition, CMs did not trigger bacteriophage induction and Shiga toxin overexpression in *E. coli* O157:H7, suggesting that CMs may provide insight to treat infections caused by pathogens encoding toxin genes in the phage genome.

Significance: The data demonstrate that a natural antimicrobial agent CMs retains antimicrobial activity in cow uterus with uterine disease that provides encouraging solution to enhance animal and public health, especially targeting antimicrobial resistant microorganisms.

T8-07 Innovative Automated Sample Prep to Reduce the Positive Rate of Non-O157 STEC

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Introduction: Detection of non-O157 STEC is based on PCR detection of virulent associated genes (*stx* and *eae*), Top6 O-groups. The prevalence of *stx*, *eae* and O-groups associated genes in food samples leads to positive results due to the detection of genes carried by different strains. The association of the VIDAS (ESPT, Phage Technology) for the capture of O-groups of interest upstream the PCR allows a better specificity for the detection of non-O157 STEC.

Purpose: Compare the VIDAS ESPT+PCR method to commercial PCR for the detection of non-O157 STEC in food samples

Methods: 376 natural raw milk products were analyzed. This food category is known to contain various *Escherichia coli* strains harboring *stx* or *eae* genes only, leading to high positive rate with PCR methods. Twenty-five g were diluted 1/10 in BPW+Acriflavine (10mg/l), homogenized, and incubated 18h at 37°C. DNA was extracted using VIDAS ESPT1 protocol or commercial lysis kit from 800µl and 50µl of enrichment broth, respectively. DNA was analyzed by PCR screen 1 (*stx* and *eae*), and tested for O-groups (Top6) when positive for both *stx* and *eae*.

Results: 4/376 samples were tested positive for both *stx* and *eae* when DNA was prepared using VIDAS ESPT1. Those 4 samples were shown positive as well for O-groups. The commercial PCR method on crude extract shown 37 positive samples for both *stx* and *eae*; 21/37 were positive for O-groups PCR. Among those 376 samples, an O26 strain (*eae* and *stx*-positive) was detected by both methods and successfully isolated.

Significance: The VIDAS ESPT automated DNA sample preparation prior to PCR enables a reduction of about 89% of positive rate for non-O157 STEC detection, limiting the number of samples to be analyzed for O-Groups.

T8-08 Validation of a New Molecular Method for the Detection of Non-O157 and O157 STECs in Beef Products

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in numerous foodborne outbreaks. With the implementation of routine testing by the USDA of additional serotypes of STEC in beef, a validated, commercially available alternate test method is needed for the rapid detection of all regulated STEC organisms.

Purpose: The objective of this study was to evaluate the Pall GeneDisc® Plate STEC Top 7 (O157, O26, O45, O103, O111, O121 and O145), a PCR-based method for the detection of STEC in 375-g test portions of raw beef trim and ground beef in comparison to the USDA FSIS reference methods using the current AOAC-PTM guidelines.

Methods: The evaluation consisted of inclusivity/exclusivity studies, method comparison of 2 different food matrices (ground beef and beef trim), and ruggedness testing. The performances of the alternate assays were compared to USDA/FSIS MLG 5B.03 (detection of non-O157 STEC) and USDA/FSIS MLG 5.06 (detection of *Escherichia coli* O157:H7) reference methods. All samples enriched and assayed with the alternate STEC Top 7 method were culturally confirmed by the alternative method confirmation procedure and the USDA/FSIS reference methods.

Results: For the inclusivity study, 50 of the 50 STEC organisms were correctly identified. For the exclusivity study, 30 of the 30 organisms were correctly called negative. For the matrix study, the statistical analysis with POD (Probability of Detection) showed the alternate method to be as good as (for *E. coli* O157 target) or better than (for the non-O157 STEC targets) the USDA reference method. The complete alternate method was also rugged against variations in incubation temperature, volume of enrichment lysed, and time of lysis of the samples with no statistical difference in the number of positive samples detected.

Significance: This new molecular method was fast and accurate for the detection of STEC Top 7 in beef meat samples.

T8-09 Development of a Novel Cross-streaking Method for Isolation, Confirmation and Enumeration of *Salmonella* from Irrigation Ponds

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Developing Scientist Competition

Introduction: The 2013 Produce Safety Rules in Food Safety Modernization Act (FSMA) require regular testing for generic *Escherichia coli* in agricultural water intended for pre-harvest contact with the edible portion of fresh produce. However, the use of fecal contamination indicators frequently does not correctly reflect distribution of foodborne pathogens such as *Salmonella enterica.* Therefore, to ensure food safety, novel methods are required for pathogens direct detection and enumeration in agricultural settings.

Purpose: We report the evaluation of different cost-effective methods for quantification, isolation, and confirmation of *Salmonella* in irrigation pond water and sediment samples.

Methods: A most probable number (MPN) dual enrichment culture method was used in combination with differential and selective agars, XLT4 and CHROMagar™ *Salmonella* plus (CSP). The necessity for PCR confirmation was evaluated, and methods were compared by cost and performance measures (i.e., sensitivity, specificity, positive predictive value, and negative predictive value).

Results: Statistical analyses showed that using XLT4 as the initial selective agar to isolate *Salmonella* colonies improved recovery compared to CSP agar; however, PCR confirmation was required to avoid false positive results on either agar. Therefore, a novel cross-streaking method utilizing CHROMagar™ agar for individual colony confirmation of *Salmonella* presence/absence on XLT4 was developed. This method classifies the colony as positive if typical *Salmonella* morphology is observed on both agars. Statistical analysis showed that this method was as effective as PCR for species confirmation of pure individual strains isolated from enrichment cultures (sensitivity = 0.99, specificity = 1.00, relative to PCR).

Significance: This method offers a cost-effective alternative to PCR that would increase the capacity and sensitivity of *Salmonella* evaluation.

T8-10 Development and Evaluation of an Integrated System for Screening and Confirmation of *Salmonella* in Food and Environmental Samples

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Introduction: Rapid methods for detecting food pathogens are fast, specific and sensitive but are considered presumptive and require additional testing to confirm positive results. In light of recent molecular advances it might be time to challenge traditional thinking and move toward acceptance of multiple molecular methods to screen and confirm food samples for presence of pathogens.

Purpose: To determine if two different but complementary molecular methods could be designed to work efficiently together and act as confirmation of one another for the detection of foodborne pathogens.

Methods: PCR assays were designed and the best assays were selected based on inclusion/exclusion testing and efficiency studies. Two independent PCR assays were designed for *Salmonella* species, a real-time PCR assay against *apeE* and an end-point PCR assay against *invA*. Initially, real-time PCR was performed on the 7500Fast instrument and end-point amplicons were hybridized to an array and detected using standard colorimetric methods. Food samples were enriched according to standard protocols and DNA was isolated using the PrepSEQ™ Nucleic Acid Extraction Kit. Subsequently, DNA extraction, real-time PCR and endpoint PCR was automated using a prototype sample-to-answer system.

Results: Real-time and end-point PCR were coupled in a single reaction tube and tested for *Salmonella* species. Each method included a separate internal positive control. The real-time PCR assay demonstrated detection of 100 inclusion strains and no detection of 30 exclusion strains, whereas the end-point PCR assay demonstrated detection of 30 inclusion strains and no detection of 20 exclusion strains. The level of detection was less than 100 genomic equivalents. The results show proof of principle for a novel sample-to-answer system that includes sample prep, real-time PCR and end point PCR detection steps.

Significance: Performing screening and confirmation together in an automated workflow provides greater ease of use and confidence to food safety labs.

T8-11 Lab and Field Performance of an Enrichment-free *Listeria* Environmental Assay

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Introduction: Environmental monitoring for *Listeria* is an essential and growing component of food safety. Existing *Listeria* environmental tests require enrichment steps that multiply the number of potential pathogens and require lengthy waits between sample and result.

Purpose: Sample6 has developed and commercialized a revolutionary technology for the detection of *Listeria* in the environment that radically reduces the time to result by eliminating the need for enrichment.

Methods: With our simple and accurate AOAC-certified Detect-L test, customers are able to take advantage of results within a single 8-hour shift.

Results: Here we detail the basic science that underlies the assay and provide details of the performance of the Detect-L test in real-world conditions.

Significance: We also discuss improvements to the Detect-L assay that further enhance time to result performance while maintaining sensitivity, specificity and robustness.

T8-12 Evaluation of a PGM-binding Method for the Discrimination of Infectious and Non-infectious Norovirus Following Inactivation with Heat or a Levulinic Acid Plus Sodium Dodecyl Sulfate Sanitizer

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Developing Scientist Competition

Introduction: Human noroviruses (HuNoV) are a major source of foodborne illnesses worldwide. As they cannot be cultured *in vitro*, alternative methods that discriminate between infectious and non-infectious HuNoV are needed.

Purpose: This study evaluates binding of GI.1 and GII.4 HuNoV to histo-blood group antigens expressed in porcine gastric mucins (PGM) as a surrogate for detecting infectious virus following treatment with heat or a levulinic acid plus sodium dodecyl sulfate (Lev/SDS) sanitizer.

Methods: GI.1 and GII.4 HuNoV (3-5 log genome copies/sample) were inactivated by heat (99°C for 5 min) or with liquid sanitizers containing 0.5% Lev/0.01% SDS (low SDS) or 0.5% Lev/0.1% SDS (high SDS) for 1 min. Treated and untreated (control) virus samples were applied to 96-well plates coated with 1 µg/ml PGM. RNase A (100 ng/well) was added to designated wells for each sample to degrade exposed RNA. The number of wells (positive/total) containing bound and potentially intact virus was calculated after real-time RT-PCR. Murine norovirus (MNV-1) inactivation was also assessed by plaque assay.

Results: After thermal inactivation, 10% (1/10) and 16% (1/6) of PGM coated wells were positive for GI.1 and GII.4 binding with RNase A treatment respectively whereas no binding (0/12) and 33.3% (2/6) binding was observed for non-RNase A treated samples in GI.1 and GII.4 HuNoV respectively. For both GI.1 and GII.4, the high SDS sanitizer completely eliminated PGM binding (0/18 each), but 18/18 wells treated with the low SDS sanitizer were positive. Murine norovirus infectivity correlated with PGM binding. All positive control wells were positive for GI.1 and GII.4 binding.

Significance: The PGM-binding method is a promising surrogate for discriminating between infectious and non-infectious HuNoVs after capsid destruction by high heat or high Lev/SDS sanitizer. Studying the kinetics of HuNoV inactivation and assay sensitivity is currently underway and necessary for further validation of the method.

T9-01 Microbiological Efficacy of an On-line Hide-on Carcass Washing Decontamination Treatment

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Introduction: Most bacteria found on beef carcasses are likely transferred from the hide during skinning operation. To reduce the numbers of bacteria on hides, an on-line hide-on carcass wash with 1.5% NaOH has been implemented at some beef packing plants. The microbiological efficacy of the treatment, however, has not been unequivocally established.

Purpose: The objective of this study was to determine the effects of the hide-on carcass wash on the microbiological condition of carcasses.

Methods: On each of 5 days, 5 samples were collected from each of the following areas on carcasses during processing: rump before hide-on wash (RBHW), rump after hide-on wash (RAHW), rump after skinning (RAS), brisket after skinning (BAS), and randomly selected areas at the end of the dressing process (EDP). The numbers of total aerobes and *Escherichia coli* were determined. A total number of 140 *E. coli* isolates recovered were subtyped using multiple-locus variable-tandem-repeat analysis (MLVA).

Results: The numbers of total aerobes recovered from RBHW, RAHW, RAS, BAS and EDP were 6.38, 4.88, 2.44, 1.97 and 2.53 log CFU/1000 cm^2, respectively. The number of *E. coli* recovered from RBHW was 5.12 log CFU/1000 cm^2. *E. coli* were not recovered from RAHW and BAS; and were sporadically recovered from RAS and EDP, with total numbers of 2.15 and 1.95 log CFU/25,000 cm^2, respectively. Substantial fractions of the *E. coli* populations from RAS and EDP were not found on the hide before the hide-on wash.

Significance: The findings show that the hide-on wash is effective for reducing the numbers of bacteria on carcasses, and *E. coli* is more susceptible to the treatment. *E. coli* is transferred onto carcass from hide during skinning as well as from sources other than the hide during the dressing process.

T9-02 Microflora on Vacuum Packaged Beef from Decontaminated Carcasses Stored at 2 or -1.5°C

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Introduction: The microbiological condition of beef produced at North American plants has been improved as a result of the use of effective carcass decontaminating treatments. The effect of these treatments on the storage life of beef has not been established.

Purpose: The objective was to determine the shelf life and the microbial flora on vacuum packaged beef from decontaminated carcasses.

Methods: Vacuum packaged bone-in and boneless cuts from decontaminated beef carcasses were assessed for their organoleptic and microbiological properties when stored at 2°C or -1.5°C for up to 160 days. Total aerobic counts (TAC) in rinse fluid from each cut were determined by plating on tryptone soya agar. Colonies recovered from fluids obtained from cuts before storage and at appropriate times during storage were randomly selected for identification of isolates by 16S rRNA gene sequencing.

Results: A storage life of 120-140 days was attained for vacuum packaged cuts of both types stored at -1.5 °C. The bone-in cuts stored at 2 °C were spoiled at earlier times. The TAC increased by 4 log units during storage to about 7.5 and 7.0 log CFU/cm^2 on cuts of either type stored at 2°C or -1.5°C, respectively. More than 20 microbial species that were mostly obligate aerobes found in soil and water were present on both types of cuts before storage. After storage for ≥ 30 days, the microflora of both cut types stored at either temperature was dominated by carnobacteria; but other lactic acid bacteria, *Lactobacillus* and *Leuconostoc* were substantial fractions of the spoilage flora at later storage times. *Enterobacteriaceae*were recovered from both types of cut at early storage times, but from only bone-in cuts at later storage times.

Significance: The findings show the longer storage life of vacuum packaged beef is likely a result of the difference in the composition of the initial microflora on beef.

T9-03 Use of Enrichment Real Time-polymerase Chain Reaction to Enumerate *Salmonella* on Chicken Parts

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Introduction: *Salmonella* that survive cooking or that cross-contaminate other food during meal preparation and serving represent primary routes of consumer exposure to this pathogen from chicken.

Purpose: To use enrichment real time-polymerase chain reaction (RT-PCR) to enumerate *Salmonella* that contaminate raw chicken parts at retail or that cross-contaminate cooked chicken during simulated meal preparation and serving.

Methods: Whole raw chickens obtained at retail were partitioned into wings, breasts, thighs, and drumsticks using a sterilized knife and cutting board. To study cross-contamination, the knife, board, and latex gloves used to partition the raw chicken were used to partition a cooked chicken breast. Chicken parts were incubated in 400 ml of buffered peptone water for 8h at 40°C and 80 rpm. After enrichment, one ml samples were used for RT-PCR and cultural isolation of *Salmonella*. In some experiments, chicken parts were spiked with 0 to 3.6 log of *Salmonella* Typhimurium var 5- to generate standard curves for enumeration by RT-PCR.

Results: Of 10 raw chickens examined, seven (70%) had one or more parts contaminated with *Salmonella*. Of 80 raw parts examined, 15 (19%) were contaminated with *Salmonella*. Of 20 cooked chicken parts examined, two (10%) were contaminated with *Salmonella*. Predominant serotypes identified were Typhimurium (71%) and its variants (var 5-, monophasic, and nonmotile) and Kentucky (18%). The number of *Salmonella* on contaminated parts ranged from one to two per part.

Significance: Results of this study indicated that retail chicken parts examined were contaminated with low levels of *Salmonella* and resulted in low levels of cross-contamination during simulated meal preparation and serving. Thus, as long as consumers properly handle and prepare this chicken it should pose no or very low risk of consumer exposure to *Salmonella*.

T9-04 Antimicrobial Effect of an Essential Oil Blend on Surface-attached *Salmonella* on Polyvinyl Chloride

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Developing Scientist Competition

Introduction: The majority of drinking water lines for broilers are made of polyvinyl chloride (PVC) and surface attachment of *Salmonella* on the inner surface of water lines can be the initial stage of biofilm development. These biofilms can be the source of *Salmonella* infection in water lines and are known to resist many antimicrobial compounds. Essential oils (EO) have antimicrobial properties and can be mixed with water at low concentrations to deactivate *Salmonella* attached to PVC surfaces.

Purpose: The objective of this study was to determine the effect of an essential oil blend on *Salmonella* attached to PVC coupon (1.8 by 2 cm tetragonal piece) surfaces compared to chlorine and untreated control.

Methods: Sterilized PVC Type 1 coupons (n = 30) were incubated in 2.2 × 10^7 CFU/ml of *Salmonella* strain cocktail (Enteritidis, Heidelberg, and Typhimurium) in TSB for 96h at 37°C at 100 rpm to develop *Salmonella* attachment. Coupons (n = 10/group) with attached *Salmonella* were treated with EO (0.05%), sodium hypochlorite (5 ppm), or deionized water, and incubated at 25°C for 24h at 100 rpm. After rinsing with PBS, coupon surfaces were swabbed using a sterile sponge premoistened with 8 ml of D/E neutralizing broth, then stomached in 50 ml of D/E neutralizing broth for 1 min at 260 rpm, and enumerated on XLT-4 plates and enriched for *Salmonella* (when direct plating was negative).

Results: There were no *Salmonella* colonies detected via direct plating and enrichment on PVC coupons treated with EO or sodium hypochlorite. The average log CFU/cm^2 (± standard error) of the control was 2.6 ± 0.34, which was significantly higher than treated groups.

Significance: The EO mixture and chlorine with water may be used to deactivate *Salmonella* attached to PVC surfaces.

T9-05 *Salmonella* Levels in Turkey Neck Skin, Bone Marrow and Spleens in Relation to Ground Turkey Production

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Developing Scientist Competition

Introduction: *Salmonella* has been detected in ground turkey at 11% although the prevalence on turkey carcasses was 2.2% in 2012. A possible route for *Salmonella* contamination of ground turkey is through neck skin and internalization in different organs such as bone marrow. Additionally, *Salmonella* presence/levels in the spleen at harvest might be an indicator for *Salmonella* contamination in ground turkey.

Purpose: The objective of this study was to determine *Salmonella* levels (prevalence and numbers) in turkey drumstick bone marrow, spleen, and neck skin samples in relation to *Salmonella*-status in the ground product.

Methods: In cooperating with a turkey production company, 15 drumsticks, spleens, and neck skins were collected per flock. The flocks sampled were either targeted or randomly selected. Target flocks were those with historically high grind *Salmonella* contamination data (i.e., >20%). All samples were tested for the presence and numbers of *Salmonella* according to USDA-FSIS selective enrichment and most probable number (MPN) methods.

Results: A total of 210 samples of each turkey part were collected and tested. *Salmonella* prevalence in neck skin, spleen, and bone was 51.0%, 5.2%, and 5.2%, respectively; whereas the means (log MPN/sample) were 2.02 (95% CI: 1.80-2.24), 1.59 (95% CI: 0.74-2.44), and 1.38 (95% CI: 1.16-1.59), respectively. *Salmonella* prevalence and numbers in spleens from target flocks were significantly higher than those from random flocks. At the flock level, when the spleen was *Salmonella*-positive, there was 80% probability that neck skin samples were also positive. Moreover, when internalized *Salmonella* MPN/g numbers were higher than zero, there was 80% probability that ground product samples were positive.

Significance: Our findings indicated that *Salmonella* was internalized in spleen and bones of turkeys at low levels. However, the contamination was at higher levels in neck skin which may pose risk toward ground turkey production.

T9-06 *Salmonella* Levels in Broiler Spleens and Ground Chicken

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Developing Scientist Competi

Introduction: According to 2012 USDA-FSIS data, the prevalence of *Salmonella* in ground chicken (28%) was significantly higher compared to that on post-chilled broiler carcasses (4.3%). *Salmonella* can be internalized into chicken internal organs, such as spleens. The grinding and mixing of *Salmonella* contaminated chicken parts can lead to cross contamination of ground chicken product.

Purpose: We hypothesized that the presence and numbers of *Salmonella* in spleen may indicate highly contaminated flocks and consequently higher levels of ground chicken contamination. The objective of this study is to determine *Salmonella* levels (presence and numbers) in broiler spleens as predictor for *Salmonella* contamination levels in ground chicken.

Methods: In cooperation with a broiler processing plant, ten composite spleen samples (after USDA inspection and evisceration) and ten 25g grab samples of ground chicken were collected per flock. The spleen outside surface was sterilized via dipping in boiling water prior to *Salmonella* testing. Ground chicken samples were tested for *Salmonella* (presence and numbers) following the USDA-FSIS methods.

Results: Two hundred and sixty spleen composite samples and 260 ground chicken samples were collected and tested for *Salmonella*. Overall, 6.9% of spleen samples were *Salmonella* positive with a mean (log MPN/ sample) of 0.94 (95% CI: 0.77-1.1); whereas 15.4% of ground chicken samples were *Salmonella* positive with a mean (log MPN/ sample) of 0.89 (95% CI: 0.71-1.06).

Significance: *Salmonella* is present at a significant level in spleen indicating pathogen internalization in broilers. *Salmonella* prevalence and numbers in ground chicken were highly variable and did not correlate with the pathogen levels in spleen. This study will be helpful to identify intervention opportunities to reduce *Salmonella* prevalence in ground chicken products.

Technical

T9-07 Reduction of *Salmonella in vitro* and on the Surface of Chicken Breast Fillets by Bacteriophage Preparation

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Developing Scientist Competition

Introduction: Lytic bacteriophages are novel, natural antimicrobials that are increasingly recognized as GRAS (Generally Recognized as Safe) for their intended use in various food products. Recently, FDA has affirmed the GRAS designation of a *Salmonella*-targeted bacteriophage preparation. USDA has approved the same preparation as safe and suitable antimicrobial for use in the production of poultry products as an incidental processing aid.

Purpose: To determine the *in vitro* efficacy of *Salmonella* phage cocktail against different *Salmonella* serotypes and the effectiveness of phage preparation in reducing *Salmonella* on chicken breast fillets.

Methods: The effect of phage cocktail on the inhibition of growth of various *Salmonella* serotypes (Enteritidis, Hadar, Heidelberg, Kentucky, and Typhimurium) was determined using a 96-well plate assay by measuring the optical density at 630 nm. Diluted overnight cultures of *Salmonella* (10^4 CFU/ml) were treated with bacteriophage at a multiplicity of infection (MOI) of 1, 100, 1000, 10000 PFU/CFU and incubated at 37°C for 48h. The efficacy of phage on reducing *Salmonella* at 4°C in TSB was determined by treating *Salmonella* cultures (10^6 CFU/ml) with phage (MOI of 100, 1000, 10000) for 24h. Chicken breast fillets inoculated with a three-strain cocktail of *Salmonella* (Enteritidis, Heidelberg and Typhimurium ~10^3 CFU/g) were treated with phage concentrations of 10^7 PFU/g and stored up to 10d at 4°C.

Results: Bacteriophage preparation at 10000 MOI completely inhibited growth of *Salmonella* at 37°C for 48 hours. At 4°C, bacteriophage preparation caused ca. 4.5 log reduction of *Salmonella* up to 24h. On raw chicken breast fillets, phage application caused 1.2, 1.3, 1.8, and 1.7 log CFU/g reductions of *Salmonella* as compared to control on d 0, 1, 7, and 10 of storage at 4°C, respectively. All reductions were statistically significant ($P \leq 0.05$).

Significance: These findings indicate that the bacteriophage preparation is effective in reducing *Salmonella* on chicken breast fillets. This phage cocktail could be an effective tool for reducing *Salmonella* on chicken parts during processing.

T9-08 Genotypic and Phenotypic Characterization of *Salmonella enterica* Serovar Typhimurium Phage Type 135 Variants and Their Survival in Poultry Processing

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Introduction: In Australia, a variant of *Salmonella enterica* serovar Typhimurium phage type 135 (locally referred to as 135a) has been associated with multiple outbreaks of human gastroenteritis involving poultry-related products. The exact reasons for the high prevalence of STm 135a on poultry meat are unknown. Certain cell surface structures, such as hydrophobic thin aggregative fimbriae (GVVPQ), are known to promote colonization and adherence in a range of hosts and resistance to environmental stressors.

Purpose: To examine the genotype of STm 135a isolates and the relationship between their phenotype and survival in the processing environment, particularly resistance to sanitisers used in commercial poultry production.

Methods: Thirty-six broiler chicken isolates of STm 135a from across Australia were typed by multi-locus variable number tandem repeat analysis (MLVA) and screened for Congo red binding and aggregative colony morphology (T medium). The ability to produce GVVPQ fimbriae was evaluated by detection and sequencing of a fragment of *agfA*, the structural gene for SEF 17 fimbrin. The survival of the isolates in simulated immersion chiller conditions to various antimicrobials (6 ppm free chlorine, 50 ppm peracetic acid and 1% v/v lactic acid) was also investigated by a microdilution method.

Results: All strains examined were representative of five different MLVA types. Further, all isolates expressed fimbriae (F^+), although production varied depending on incubation temperature. The *agfA* gene was detected in all isolates and sequence analysis revealed ≥ 98% similarity. STm 135a cells, whether fimbriated or not, were readily injured within 10 mins by working concentrations of sodium hypochlorite and peracetic acid. When chicken skin was added to the chiller system, survival increased.

Significance: As a direct relationship between fimbriation of STm 135a and survival in simulated chicken processing was not evident in this study, other ecological factors should be considered in future studies.

T9-09 Water Recycling and Energy Recovery in Commercial Poultry Processing - Spin Chiller Wastewater Treated Using a Ceramic Membrane System

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Developing Scientist Competition

Introduction: Specific unit operations in poultry processing plants use large quantities of water and energy, with little or no recovery. With increasing consumption of chicken meat, processors need to increase production in order to meet demand, while managing limitations on water consumption and discharge. This, along with the introduction of a carbon emissions trading scheme in Australia and globally, has driven investigation of recycling water and energy to maximize processing efficiency.

Purpose: To determine if spin chiller wastewater can be recycled, using membranes, while maintaining quality and safety, and to quantify the potential reduction in water and energy consumption and the consequent environmental impact.

Methods: A pilot plant, utilizing a 0.2 µm α-Al_2O_3 ceramic membrane, was used over twelve months to treat raw spin chiller wastewater via membrane filtration. Trials (4 hours to 7 days) were conducted to gather membrane performance data as well as concentrate and filtrate samples. The concentrate and filtrate were analysed quantitatively for total viable count (TVC), total coliforms (TC), and *Escherichia coli*, total suspended solids (TSS), turbidity, pH and free chlorine, and qualitatively for *Salmonella*.

The results were used to model the benefits of full scale operation using Life Cycle Analysis (LCA) software (SimaPro), assessing overall impact on a variety of environmental factors including climate change impact, resource depletion, eutrophication and ecotoxicity.

Results: *E. coli* and *Salmonella* were not detected in any filtrate samples (<1 MPN/ml). TVC was reduced by 2-4 logs. All water quality indicators (TSS, pH and turbidity) met Australian and major international water recycling guidelines. LCA showed that full scale implementation of ceramic membrane water recovery would be beneficial to commercial poultry processing in terms of climate change impact, resource depletion and eutrophication.

Significance: Spin chiller wastewater can be treated to allow recycling and energy recovery, reducing the environmental impact and operating cost of chicken meat processing.

T9-10 Evaluation of Chicken Meat Juice on Hands, Chicken Packages and Contact Surfaces during and after Grocery Shopping

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Introduction: Raw meats are potential sources of foodborne pathogens and can contaminate prepared foods through direct or indirect contact during food handling and preparation.

Purpose: We have developed and validated an immunoassay for quantitative detection of chicken meat juice on hands and various food contact surfaces and evaluated the contamination and transferring of meat juice from chicken packages to hands and contact surfaces during and after grocery shopping.

Methods: An immunoassay was developed using a monoclonal antibody to a muscle protein, troponin I. Swab procedures were designed to detect meat juice on hands and food contact surfaces. Consumer shopping studies were conducted in three cities. A total of 394 swabs were collected from 96 participants. The swabs were taken from: (1) consumers' hands at store after they touch three items and have left the meat section, (2) the outside of the poultry packages, (3) the inside of grocery bags if the customer does not throw away the grocery bag, (4) the items the poultry touched in the cart, and (5) the surface that the poultry touched at the home.

Results: The results of meat juice assay showed, among 96 participants, 47 (49%) had detectable meat juice (from 1 µl to more than 500 µl) on the chicken packages they purchased. Among these participants (47), 16 (34%) had detectable meat juice (between 1 µl and 50 µl) on their hands, 10 (21%) had detectable meat juice (between 1 µl and 500 µl) on the grocery bags, 12 (26%) had detectable meat juice (between 1 µl and 500 µl) on countertop or refrigerator surfaces, and 23 (49%) had detectable meat juice (between 1 µl and 50 µl) on other food items.

Significance: The data indicated that there is a high chance of contamination and transfer of meat juice from chicken packages to hands and other food contact surfaces.

T9-11 Longitudinal Tracking of *Listeria monocytogenes* Persistence in Meat Processing Facilities before and after Employee Trainings, Behavioral Changes, and Facility Improvements

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Developing Scientist Competition

Introduction: Meat and poultry products have been associated with several multi-state outbreaks of foodborne listeriosis. Studies which employ combined testing and molecular subtyping to define *Listeria monocytogenes* persistence in meat processing establishments, and identify mitigation strategies, including employee trainings, behavioral changes, and physical facility improvements, are limited.

Purpose: This study tracked *L. monocytogenes* contamination patterns for six months in two meat processing facilities. Employee trainings, suggested mitigation strategies, and behavioral assessments were provided. Follow-up sampling was conducted to assess the impact of these control measures.

Methods: Sponge samples were collected monthly from environmental sites and products, and processed according to USDA-MLG protocol 8.07 with modifications. *Eco*RI ribotyping was used as the subtyping method for *L. monocytogenes* isolates. Assessments of employee knowledge and self-reported behaviors were analyzed via a Paired *t*-Test and a McNemar's Test, respectively. Suggested facility physical changes were implemented. Follow-up samples were collected on two occasions using identical testing methodology and subtyping.

Results: Statistical analyses identified persistent *L. monocytogenes* strains in Facilities 1 and 2. Employee knowledge assessment score means increased for both facilities post-training; Facility 2 knowledge increased significantly ($P < 0.05$). Facility 1 implemented physical facility improvements post-training, and significant behavior changes were reported. Follow-up sampling demonstrated that *Listeria* prevalence decreased significantly ($P < 0.0001$) although ribotype DUP-1042B persisted. Facility 2 reported one significant behavior change, did not immediately implement physical improvements, and *Listeria* prevalence remained at 44.1% for follow-up sampling 1. The facility's lowest point prevalence (11.8%) followed implementation of physical improvements between follow-up samplings 1 and 2.

Significance: A combination of testing, molecular subtyping, employee trainings, and physical facility improvements may aid in mitigation of *L. monocytogenes* contamination. However, outcomes may vary by facility and persistent subtypes may withstand stringent control measures that are implemented.

T9-12 Determination of the Thermal Inactivation Kinetics of O157:H7 and Non-O157:H7 *Escherichia coli* in Turkey Deli Meat and Ground Beef

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Developing Scientist Competition

Introduction: In 2012, USDA declared six non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) as food adulterants and included them in the zero-tolerance policy. This created a need to determine the thermal inactivation kinetics for these pathogens so as to be able to design adequate thermal processes.

Purpose: The objective of this study was to determine the thermal inactivation kinetic parameters for non-O157 STEC strains and compare them to O157:H7 STEC on raw and fully-cooked meat products.

Methods: Five strains of individually grown O157:H7 or non-O157:H7 STEC were combined into two separate cocktails containing 8 log CFU/ml. Inocula were spread onto the surface of a 3.8 cm diameter circle of turkey deli meat or mixed with ground beef. Both samples were held for 30 min and then placed into vacuum-sealed polyethylene-nylon bags. Bags were submerged in a circulating-water bath at 56°C, 58°C or 60°C for various times, removed and cooled in an ice bath. Products were blended, serially diluted, and samples spread-plated on tryptic soy agar plates. Surviving bacteria from three replicate experiments were enumerated after 24h incubation at 37°C. D- and z-values were calculated using linear regression.

Results: In deli meat at 56, 58 and 60°C, D-values were 15.42 ± 0.08, 5.23 ± 0.16 and 1.89 ± 0.27 min for O157:H7 STEC, and 15.48 ± 1.0, 7.20 ± 0.56 and 2.86 ± 0.22 min for non-O157:H7 STEC, respectively. In ground beef at 56, 58 and 60°C, D-values were 11.85 ± 0.93, 5.66 ± 0.05, 1.55 ± 0.04 min for O157:H7 STEC, and 10.59 ± 1.59, 3.30 ± 0.25, 1.39 ± 0.29 min for non-O157:H7 STEC, respectively. z-values for O157:H7 and non-O157:H7 STEC in deli meat were 4.39 ± 0.31 and 5.46 ± 0.14°C, respectively, and in ground beef 4.54 ± 0.24 and 4.54 ± 0.15°C, respectively.

Significance: Composition of food influences the thermal inactivation parameters for O157:H7 and non-O157 *E. coli.* These data will help in designing adequate thermal processes to inactivate pathogenic *E. coli* in meat products.

T10-01 Characterization of Phage from Environmental Water Samples and the Potential of Phage Tailspike Protein (TSP) in Bacteria Detection

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Developing Scientist Competition

Introduction: The high abundance of phage in the environment and their specificity with the bacteria host make them an excellent tool for bacteria detection.

Purpose: To characterize phage specific to *Salmonella* and *Escherichia coli* from water samples in the Southeastern Michigan and to develop a novel bacteria detection method using phage.

Methods: Environmental water samples were collected from rivers and waste water treatment plant (WWTP) in Southeastern Michigan. *E. coli* and *Salmonella* phage were isolated from water samples using a soft agar overlay technique with multiple indicator strains. Host range of phage was determined using 18 *Salmonella* strains and 14 *E. coli* strains. Transmission Electron Microscopy (TEM) was used to visualize the morphology of representative phage. Tailspike Protein (TSP) from *E. coli* O157 phage ΦV10 was functionalized on a graphene field-effect-transister (FET). The binding of TSP and *E. coli*O157 was measured by the shift of Dirac Point.

Results: A total of 70 *Salmonella* phage and 33 *E. coli* phage were purified. Twelve *E. coli* phage were able to grow in *E. coli* O157. All *Salmonella* phage comprising of 37 host range patterns were able to multiply in 3 or more indicator strains. Out of 9 host range patterns observed in *E. coli* phage, 6 patterns of phage were able to multiply in 3 or more indicator strains. This suggests a broad host range of the phage recovered. TEM demonstrated tail structures in most *E. coli* phage and *Salmonella* phage tested. Detectable conductance change was observed when *E. coli* O157 binds to TSP-functionalized graphene FET whereas *Salmonella* binding only caused very small shift in Dirac Point, indicating high specificity of *E. coli* O157 interaction with the TSP.

Significance: Phage with broad host ranges are prevalent in water. Phage TSP has the potential to be applied to bacteria detection.

T10-02 Development and Validation of a Multi-organism/Multi-application Certified Reference Material

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Introduction: ISO Guide 17025 requires use of CRMs or secondary reference materials to monitor validity of all tests.

Purpose: To determine if multi-organism/multi-application reference materials could be produced and certified according to ISO Guide 34 with adequate stability, precision and performance to be used as received for commonly performed selective media methods in food safety laboratories.

Methods: *E. coli (NCTC9001), K. aerogenes (NCTC8167), S. aureus (NCTC6571)* and *C. albicans (NCPF3255)* were combined and preserved by dehydration at activity levels targeted to produce countable colonies at approximately the mid-point of 7 AOAC approved rehydratable film test methods(Methods 990.12, 991.14, 2001.05, 997.02, 2003.11). Approximately 1000 individual samples were prepared from the formulation lot. Twenty samples were selected at random for internal certification by the listed methods. Twenty ISO Guide 17025 accredited laboratories were chosen for a 3rd party inter-laboratory proficiency testing study using the listed methods. Archived samples were tested for stability as well as alternative method performance. Concurrently, single organism controls were prepared and validated similarly. Food matrix impacts were further investigated.

Results: Results of internal certification and inter-laboratory proficiency testing were statistically evaluated to determine value assignments and overall performance. Expanded uncertainties of each value assignment were determined as per ISO Guide 34 and were calculated to be about +/-10%. The %RSD of the inter-laboratory proficiency testing data with n = 20 was less than 8.8%. Mixing organisms into a single sample showed no negative effect on performance relative to single organism formulations. Stability of the CRM exceeds 8 months with testing continuing.

Significance: The performance data indicates that stable, fit for use multi-organism/multi-applications CRMs can be developed and produced.

T10-03 Assessment of *Pseudomonas fluorescens* and *Escherichia coli* O157:H7 Populations in Monocultures and Co-cultures as Influenced by Substrates, Temperatures and Storage Time

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Introduction: *Escherichia coli* O157:H7 and other foodborne pathogens are major sources of contamination of minimally processed leafy greens. Assessment of bacterial populations and growth in liquid cultures may enhance quantification and prediction of microbial interactions for biological applications.

Purpose: Assess the effects of storage temperatures and time of incubation on interactions of bacteria under diverse nutrient conditions and evaluate the effect of relative ratios of *Pseudomonas fluorescens* to *E. coli* O157:H7 on survival and growth of *E. coli* O157:H7 population.

Methods: Bacteria populations were assayed in monocultures and co-cultures in sterile distilled water (SDW), buffered peptone water (BPW), and trypticase soy agar (TSA) following incubation at temperatures of 5-37°C and storage times of 0 (control), 24 and 48 hrs.

Results: In co-cultures, *E. coli* O157:H7 cell counts were 1.71-6.27 (20°C) and 1.90-9.03 log CFU/ml at 35°C. Similarly, populations of *P. fluorescens* in co-cultures ranged from 3.09-5.91 and 2.88-8.36 log CFU/ml at 20 and 35°C, respectively. As expected, the populations varied with bacterial strains, storage temperatures and time, and growth substrates; and were significantly ($P < 0.05$) lower on SDW and BPW than on TSB. Populations of both bacteria were significantly ($P < 0.05$) lower at 5°C than at temperatures of 20 or 35°C. Low suppressive activity of *P. fluorescens* against *E. coli* O157:H7 were recorded and varied with pathogen strains.

Significance: The biological control implications of the experiments suggest that *P. fluorescens* co-cultured with *E. coli* O157:H7 can be used to assess microbial interactions. This may also be utilized to quantify the biocontrol potential of *P. fluorescens* on *E. coli* O157:H7 on produce during transportation of leafy greens or abusive temperature conditions. The potential for suppressive activity should be assessed on produce to optimize biocontrol efficacy.

T10-04 Using Physiological Growth Parameters to Predict Spore-forming Bacteria Behavior All Along Their Life Cycle as Vegetative Cells or Spores

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Introduction: Spore-forming bacteria in food are a major cause of food poisoning or food spoilage, leading to a heavy burden. Empirical models have been developed to predict vegetative cells and spores behavior but very few integrate parameters with physiological meaning.

Purpose: The aim of this work is to model *Bacillus weihenstephanensis* KBAB4 (*B. cereus* group) and *Bacillus licheniformis* AD978 behavior during sporulation, spores germination and growth after a heat-treatment, using one single set of parameters: T_{min}, T_{opt}, T_{max} and pH_{min}, pH_{opt}, pH_{max}.

Methods: Effects of temperature and pH on growth of vegetative cells, their ability to produce spores and the spores' germination and activity recovery capacity after a heat-treatment have been quantified. For every phenomenon, the effect of temperature was quantified from 4°C to 40°C for *B. weihenstephanensis* and from 15°C to 60°C for *B. licheniformis* and the pH was studied from 4.5 to 9.5 for both strains. For the sporulation and the spores recovery, heat-resistances at 85°C, 90°C, 95°C for *B. weihenstephanensis* and 95°C, 100°C, 105°C for *B. licheniformis*, were estimated using a Bigelow-like model.

Results: Optimal growth temperature was around 31.9°C for *B. weihenstephanensis* and at 49.0°C for *B. licheniformis* and optimal pH was between 7.5 and 8.0 for both strains. Sporulation and recovery conditions range are circumscribed in growth conditions field (temperature and pH). Models for each phenomenon have been developed, based on modular mathematical approach, and only one set of values (minimal, optimal and maximal growth temperature and pH) with biological significance are integrated as parameters.

Significance: These models can be easily used to identify process conditions yielding microbial hazard related to spore contamination and predict spore forming bacterial behavior using physiological parameters, largely available in literature.

T10-05 Microbial Levels and Fecal Contamination Indicators in Restaurant Salads: Correlations between Salad Type, Restaurant Ownership and Customer Traffic Volumes

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Introduction: Food workers are responsible for approximately 20% of foodborne outbreaks.

Purpose: The objective of this study was to understand the levels of bacterial levels and fecal contamination indicators in specialty salads and leafy green salads as a function of restaurant ownership (corporately owned versus independently owned), salad types (leafy green versus specialty salads) and customer (low traffic versus high traffic) volumes.

Methods: The salads (100 specialty and 100 leafy green salads) were obtained from 5 corporately owned (CO) restaurants and 5 locally owned (LO) restaurants during periods of high customer traffic and low customer traffic. Of these, 50 specialty salads and 50 green leaf salads were obtained from high and low customer traffic periods and screened for aerobic bacterial counts (APC), coliforms, *Escherichia coli*, *Enterococcus* spp. somatic coliphages, and male-specific coliphages.

Results: *E. coli*, *Enterococcus*, male-specific and somatic coliphages were present in 19%, 78.5%, 20%, and 22.5% of the restaurant salad samples, respectively. Indicator organisms were higher in specialty salads compared to leafy green salads. Overall, restaurant type was shown to influence the concentration of indicator organisms in restaurant salads. Two-way ANOVA analysis showed that salads from LO restaurants had significantly (P = <0.05) higher concentrations of APC, *Enterococcus,* and male-specific coliphages than salads purchased from CO restaurants. Total coliforms and somatic coliphages were significantly (P = <0.05) higher in CO restaurants. Two-way ANOVA analysis found that there was no significant differences observed for the concentrations of APC, total coliforms, *E. coli*, *Enterococcus*, and male-specific and somatic coliphages for salads purchased during different volumes of customer traffic.

Significance: These results imply that salads especially specialty salads, can be a vehicle for fecal contaminants and that microbiological quality within corporately owned and locally independently owned restaurants is approximately the same. These results can assist the restaurant industry understand the potential vulnerabilities in their practices.

T10-06 Estimating Significant Differences in Serotype Prevalence from *Salmonella* Survival Studies Using a Four-serotype Cocktail in Low-a_w Whey Protein Powder at Different Temperatures and a_w

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Introduction: The use of serotype cocktails is standard for inactivation studies to collect data on the worst-case scenario. Statistically significant differences in serotype survival can be obtained through rapid screening and statistical analyses. Regression models associate a response with a group of fixed effects. One can also use a multinomial mixed effects logistic model including random and fixed treatment effects. A non-parametric alternative is a chi square test comparing observed and expected values under the assumption that study conditions are not related to serotype prevalence.

Purpose: The aim of this study was to evaluate the suitability of three statistical approaches in estimating significant differences in the prevalence of four *Salmonella* serotypes in low-a_w whey protein powder.

Methods: Whey protein powder was equilibrated to a_w 0.18 and 0.54, inoculated with a cocktail of *S*. Agona, *S*. Tennessee, *S*. Montevideo and *S*. Typhimurium and stored at 36°C for six months and 70°C for 48 hours. PCR Multiplex serotyping was used to identify 306 randomly picked isolates. A multiple linear regression model, a multinomial mixed logistic model and a chi square test were used to identify significant differences in serotype relative prevalence.

Results: Regression models and the chi square test were not appropriate, as data were not normally distributed and observations were not independent. The most suitable model was the multinomial mixed logistic model accounting for variability among replications and the nature of the data. *S*. Tennessee showed to survive better than *S*. Montevideo and *S*. Typhimurium at higher temperatures and higher a_w levels ($P < 0.05$). The relative prevalence of *S*. Agona to *S*. Tennessee did not change significantly with increasing temperature ($P = 0.211$) or a_w ($P = 0.453$).

Significance: The statistical approach presented in this study may be applied to future survival studies that employ serotype cocktails.

T10-07 Alive and Well in Low-moisture Conditions - What We Can Learn about *Cronobacter sakazakii* Using RNA Sequencing (*RNA-Seq*) and Transposon-directed Insertion Site Sequencing (TraDIS)

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Developing Scientist Competition

Introduction: *Cronobacter* species are opportunistic pathogens, associated with serious illness in neonates. Powdered infant formula (PIF) has been epidemiologically linked to infections. Little is known about the mechanisms *Cronobacter* employ to survive and persist in low-moisture environments, including PIF production.

Purpose: To determine the genetic signals contributing to the survival and persistence phenotype in low-moisture environments.

Methods: *Cronobacter sakazakii* SP291, a persistent PIF factory isolate, was selected. Early stationary phase cells were dried onto industrial grade stainless steel coupons for 4h at 24°C, to simulate a low-moisture environment. Liquid culture was used as control. Total RNA was purified for RNA sequencing (*RNA-seq*) and sequences were mapped to the reference genome. A transposon-mutant library was constructed in *C. sakazakii*SP291. Pools of random insertion mutants were desiccated as before. The mutant library was screened by transposon-directed insertion site sequencing (TraDIS) and compared against the original, to identify genes involved in low-moisture survival.

Results: Absolute and relative levels of gene expression were determined using the *transcripts per million* (TPM) method, applying a $\log_2$-foldchange cut-off value of 3. A total of 4,177 genes (99.9%) were expressed in the RNA pool, with 107 genes (2.56%) being upregulated and 22 genes (0.53%) downregulated in low-moisture conditions. The upregulation genes included the osmotic stress response genes *betA*, *betB*, *betI*, *betT*, *proX*, *proW*, and *opuCB*; the heat-shock response gene *rpoH*; oxidative stress response gene *osmC*, among others. Downregulation genes included the anti-RssB factor gene and several hypothetical genes of unknown function. Comparative analysis of the un-desiccated and the desiccated mutant pools following TraDIS confirmed the *RNAseq* data. Furthermore, qRT-PCR validated a selected sub-set of these gene targets, thereby confirming both approaches. A model describing the transcriotomic response of *C. sakazakii* SP291 is presented.

Significance: This is the first report combining *RNA-seq* and TraDIS to study gene expression in *Cronobacter*. Results show the bacterial response at the transcriptional level in low-moisture conditions. These findings can be used to assist managers in developing guidance measures to reduce the risk of *Cronobacter* contamination in PIF and production environments.

T10-08 Real-time PCR Methods for Detection of Crustacean Shellfish Allergens in Challenging Food Matrices and Processing Conditions

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Introduction: Food allergen detection requires methods that are highly sensitive and specific for the target allergen. For crustacean allergen detection, it is also important that methods be able to distinguish the different types of crustaceans—shrimp, crab, and lobster—from each other. Real-time PCR meets all of these requirements.

Purpose: The work described here was carried out to evaluate real-time PCR methods we developed for shrimp and lobster. We used food matrices with inherently low DNA content, acidic pH, and treatment with high temperature and pressure.

Methods: Group-specific real-time PCR primers and probes were developed for shrimp and lobster using mitochondrial 12S and 16S gene sequences. Shrimp and lobster meat were spiked into food matrices at 0.1, 1, 10, 100, 1000, 10^4, and 10^5 ppm, homogenized, and subject to DNA extraction and PCR using methods optimized for this work.

Results: Results were used to generate standard curves which were analyzed with respect to linear range, statistical R^2 value, and PCR reaction efficiency. In neutral pH and untreated acidic conditions, both shrimp and lobster assays had reaction efficiencies of 94%-112%, lower limits of 0.1-1 ppm, and linearity over 6-8 orders of magnitude. Treatment with high temperature and pressure in an acidic food matrix resulted in a loss of signal.

Significance: There is no effective treatment for food allergy. Food allergic people can react to minute amounts of allergen, so they rely on avoidance of offending foods. This makes sensitive detection methods imperative in protecting the public health. With the exception of combined high heat, high pressure, and acidic pH, the method is highly sensitive and robust.

T10-09 Degradation of Patulin in Apple Juice Products by Ultraviolet Light of Different Wavelengths in UVC Range

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Developing Scientist Competition

Introduction: Ultraviolet (UV) irradiation is a non-thermal technique that can be applied for the photo-degradation of patulin, a mycotoxin produced by several species of fungi involved in spoilage of fruit and found in fluid and solid apple products.

Purpose: The study evaluated three UVC wavelengths (222 nm, 254 nm and 282 nm) to degrade patulin with absorption maximum at 276 nm introduced into apple juice or apple cider.

Methods: All UV processing conditions were performed in triplicate (n = 3) with completely independent and randomized design using bench scale pathogen reduction box.

Results: The average UV fluences of 19.6, 84.3, 55.0 and 36.6 $mJ \cdot cm^{-2}$ resulted in the 90% reduction of patulin in apple juice through the exposure of UV lamps at 222, 254, 282 nm wavelength and the combination, respectively. Therefore, the efficiency order of three wavelengths was: 222 nm > 282 nm > 254 nm. In terms of color, treatment of apple juice with 222 nm resulted in an increase in L* value but decrease in a* and b* values although the changes were insignificantly different ($P > 0.05$) to non-treated controls based on a sensory evaluation. The ascorbic acid loss in juice treated at 222 nm to support 90% reduction of patulin was 36.5%. This compares to 45.3% and 36.1% ascorbic acid loss with samples treated at 254 nm and 282 nm. The current work demonstrated that the 222 nm wavelength possesses the highest efficiency on patulin reduction in apple juice compared with the 254 nm and 282 nm with no benefit gained from using a combination of wavelengths.

Significance: The advantages of no significant changes in pH, total soluble solid and color changes in apple juice after UV exposure at 222 nm for a time to degrade 90% of patulin (T_{90}) demonstrate potential of further development of this novel UV source for the commercial applications.

T10-10 Influencing Food Safety Behavior within the Food Processing Sector by Means of a Knowledge Transfer Program: A Case Study

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Introduction: Food safety behaviour is an area of growing interest and of concern on a global basis. A key to the effective control of behaviour is the development of technical controls, education of operators and verification and validation of food handling practices.

Purpose: The methods for embedding good food safety behaviour are complex and varied. This study aimed to evaluate a specific knowledge transfer model on the practices within 32 private food sector businesses.

Methods: A structured knowledge transfer model was developed to facilitate the embedding of food safety knowledge from a learning provider into the private sector food business via a suitably qualified food graduate. A four phase food safety management programme was used as the benchmark. Fifty such programmes were run in 32 companies. Ten food companies were interviewed at Director level to establish impact on performance.

Results: Sampling of food safety monitoring controls showed 88% of companies experienced increases in the measurement of food safety controls within their process. The intervention also resulted in a 16% reduction in the number of missed food safety measurements (e.g., CCP at set time intervals). Thirty per cent of companies gained new 3rd party accreditation with 90% of the sample companies indicating that the 4 phase structure employed in embedding food safety practices and behaviour had been critical to improved technical performance. Prior to the introduction of the phased programme, which included the definition of systems / controls, training, Internal Audit (via verification) and validation (reverent), the area for largest non-conformances was found to be in the training phase of the programme. This will be discussed.

Significance: Impacts of the study are wide ranging from behavioural change through a clearer understanding and accountability to commercial advantage gained through 3rd party accreditations. Fifteen companies gained BRC Global Food Standard and sales rose by $60,000,000 as a result of improved technical performance and increased customer confidence.

T10-11 Survey of Internal Temperatures of Lebanese Domestic Refrigerators and Analysis of Factors Affecting Them

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Introduction: Lebanon is currently suffering the worst electrical power crisis in its history, which is negatively affecting the internal temperatures in domestic refrigerators and putting the Lebanese population at a higher risk of foodborne illnesses.

Purpose: A survey was carried out from February to November 2013 and internal temperatures were recorded in domestic fridges using data loggers. In addition, socio-economic status of the households, conditions of the refrigerators and the power supply were analyzed.

Methods: A short questionnaire was administered to participants, enabling the following information to be obtained: characteristics of the family, characteristics of the power supply, characteristics of the refrigerator and the use conditions. One hundred and forty-seven domestic refrigerators were surveyed in the three main cities (Beirut, Tripoli and Saida) every 5 min over a period of 72 hours.

Results: The age of 30% of the surveyed fridges exceeded 13 years, 26% were fully loaded, 16% had bad door seals, 100% did not have an internal thermometer, 20% had a heat source less than one meter close and 79% of participants reported that their refrigerator is not always connected to power. The temperatures of the surveyed refrigerators were: average 8.0°C, minimum -5.9°C and maximum 37.0°C. A significant proportion (71%) of the refrigerators had a mean temperature >6°C. Statistical analysis showed that there was a significant ($P < 0.05$) difference between mean temperatures of the three days in refrigerators in the three locations. The socio-economic status of the households (income and number of family members), the refrigerator status (age, brand name, load level and seal status) and the power supply characteristics (frequency of governmental power cuts and availability of alternative power) had no significant ($P > 0.05$) effect on internal temperature distribution of the refrigerators.

Significance: The temperature data collected by this survey can indicate the amount of time that refrigerators have an internal temperature above a minimum bacterial growth temperature. In addition, these data can assist with food safety promotion and act as an input into food safety risk assessments.

T10-12 Food Safety and Handling Knowledge and Practices of Lebanese University Students

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Introduction: Information about Lebanese university students in terms of their food safety knowledge and practices has not been studied previously.

Purpose: The aim of this study was to assess the level of food safety handling knowledge and practices among 1172 Lebanese university students (mean age 20.0 ± 1.6 SD) and to explore the association between their knowledge/practices and the socio-demographic and academic characteristics.

Methods: Participants were undergraduate students enrolled in the Lebanese American University from different majors and years of study, from different areas of residence, living alone or with others, and having working or non-working mothers. They completed a questionnaire of 16 food handling practices and 14 food safety knowledge questions related to preparation, cross contamination, storage and hygiene.

Results: On average, the students scored 53.6 ± 15.8% and 44.7 ± 14.3% on the knowledge and practices parts, respectively. Female students scored higher on both practices and knowledge questions than male students; however, the difference was significant ($P < 0.001$) for the practices part only. Health-related majors scored significantly ($P \leq 0.001$) better on both practices and knowledge questions. Senior students scored highest on the knowledge questions, followed by juniors, sophomores and finally freshmen and the difference was borderline significant ($P = 0.07$). The area of residence had a significant ($P = 0.006$) effect on the food safety practices questions. Students living with parents scored higher in both knowledge and practices parts than those living alone or with roommates; however, the difference was significant ($P = 0.009$) in the knowledge part only. Students who cook all the time showed a significant ($P = 0.001$) lower difference in terms of their food safety knowledge compared to those who cook less frequently. Food handling practices and food safety knowledge scores were significantly ($P < 0.001$) related with a weak to moderate correlation coefficient (R = 0.231).

Significance: Our results confirm the need for ongoing educational initiatives to improve the relatively low food awareness among the Lebanese young adults group.

T11-01 Difficulties of Spiral Freezer Decontamination: Eradicating *Listeria* spp. Using Chlorine Dioxide Gas

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Introduction: Spiral freezers are notorious for microbial contamination and being very difficult to clean because of the complex and crowded interiors. Current methods of cleaning spiral freezers (i.e., spraying, foaming and wiping) are not completely effective because they are liquid-based methods and have difficulty getting into tight spaces and hard to reach areas. A new thought is to fill the space with a sterilant gas achieving complete coverage, resulting in more effective decontamination and shorter downtimes.

Purpose: A study was initiated to validate the efficacy of chlorine dioxide gas (CD) on the inactivation of *Listeria monocytogenes* at low concentration levels and in short periods of time.

Methods: Traditional exposure for CD to achieve a 6-log sporicidal kill is 720 ppm-hours. Glass coupons inoculated with *Listeria monocytogenes* were introduced into a 17 cubic foot chamber and exposed to 50 – 720 ppm-hour decontamination cycles at 1 mg/l. The inoculated and exposed coupons were then enumerated and/or enriched along with proper controls to determine the total log reduction. Concentrations can be monitored in real time with a UV –VIS spectrophotometer to guarantee the correct exposure was achieved throughout the target area.

Results: Chlorine dioxide gas achieved greater than a 5-log reduction with a 400 ppm-hours exposure on glass coupons. A case study involving a 20,000 ft^3 spiral freezer that was contaminated with *Listeria spp*. was successfully decontaminated using chlorine dioxide gas resulting in no positive swabs for up to 6 weeks after exposure.

Significance: Spiral freezers are very difficult to clean and therefore make it hard to prevent microbial contamination. Traditional methods for cleaning spiral freezers (i.e., spraying, foaming and wiping) are not completely effective because they are liquid-based methods and have difficulty getting everywhere. Filling the space with a sterilant gas and achieving complete coverage results in a more effective decontamination.

T11-02 Persistent *Listeria monocytogenes* Strains from Retail Delis Have Increased Likelihood of Forming Biofilms and Tolerating Sanitizers

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* (LM) is commonly found in retail deli environments, which may result in cross-contamination to foods during normal operations. Previous studies by our group found that the same strain of LM (based on Pulsed Field Gel Electrophoresis (PFGE) subtyping) can persist in a deli for over a year even if it is routinely cleaned and sanitized.

Purpose: The aim of this study was to determine if strains from retail delis with evidence of persistence, compared with transient strains, are more likely to form biofilms and/or have increased tolerance to commercial sanitizers.

Methods: A total of 41 persistent (isolated from the same site and store with the same PFGE pattern for ≥2 months) and 97 non-persistent strains were evaluated. Sanitizer tolerance was assessed by minimum inhibitory concentration (MIC) of quaternary ammonia sanitizer from [0 ppm-100 ppm] in minimal media for 24h; growth in ≥ 12.5 ppm was considered tolerant. Biofilm attachment assays were conducted in minimal media in multi-well polyvinyl chloride (PVC) plates. Cells were evaluated at 1, 3 and 5 d, washed and stained with crystal violet. Cells were suspended in 30% acetic acid followed by optical density quantification to indirectly measure attached cells; strains with average $OD_{590} \geq 0.13$ were considered to have increased biofilm formation.

Results: There was a positive correlation between persistent strains and increased ability to form biofilms on day 3 (ANOVA; $P = 0.010$). There was also a positive correlation between persistent strains and increased ability to tolerate quaternary ammonia sanitizer (ANOVA; $P = 0.016$). Further, there was a negative correlation between strains with enhanced biofilm formation on day 5 and sanitizer tolerance in nutrient rich conditions (Linear regression; $P = 0.032$).

Significance: Persistent LM strains from retail delis are more likely to have increased ability to form biofilms and tolerate quaternary ammonia. This underscores the importance of sanitation programs that can eliminate niches harboring LM, which will reduce transmission to foods.

T11-03 Employee- and Management-implemented Interventions Reduce *Listeria monocytogenes* Prevalence in Retail Delis

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Introduction: Deli meats sliced at retail are predicted to cause 83% of deli meat-associated listeriosis cases annually. While *L. monocytogenes* (LM) is commonly found in delis, environmental prevalence varies by store (0-40%). Enhanced daily SSOPs and a deep clean SSOP executed by a third-party cleaning service immediately reduced LM prevalence in delis, but reductions were often not sustained over time.

Purpose: The purpose of this study was to assess the efficacy of a deep-clean SSOP conducted by store employees and management, complemented with training and facility improvements, all aimed to reduce LM prevalence in stores with known high prevalence and persistence rates.

Methods: Fifty delis among 6 states were screened for LM using a predictive logistic regression model that estimates the probability of high LM prevalence in a deli. The model identified 13 stores with potentially high LM prevalence; 7 stores were confirmed (based on ≥2 of 20 food and non-food contact surfaces positive for LM for ≥2 months) and enrolled for further study. Retail employees, under researcher supervision, executed deep-clean SSOPs; additional interventions (e.g., facilities improvements, training, and education) were incorporated in stores. Environmental samples (n = 20) were collected immediately before and after, and for 6 months post-deep clean.

Results: Deep-cleans immediately reduced LM prevalence in 6 of 7 stores tested. A total of 22/139 (15.80%) samples before and 8/140 (5.71%) samples after deep-cleaning were positive for LM, a mean decrease of 10.1% positive rate per store (CI_{95}: -0.01, 21.08%; P = 0.066). Interventions reduced average monthly LM prevalence in each store over time by 9.7% (CI_{95}: 2.50, 16.90%; P = 0.017).

Significance: Employee- and management-executed deep-cleans complemented with training, education, and maintenance programs can reduce LM prevalence, and may play a significant role in preventing cross-contamination of RTE deli meats from the deli environment.

T11-04 Production of Antilisterial Bacteriocin by *Lactobacillus plantarum* under Different Stress Conditions

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Developing Scientist Competition

Introduction: Nowadays, bacteriocin producer lactic acid bacteria cultures are commonly used as bio-preservatives in fermented foods and numerous scientific studies examine their bacteriocins against *Listeria* spp. *Lactobacillus plantarum* is commonly used in starter cultures for fermented meats.

Purpose: The aim of this study was to investigate how *Lactobacillus plantarum* ESB 202 (isolated from fermented meat) produce bacteriocins under different stress conditions: pH, NaCl and temperature.

Methods: The modified (pH 3.5 and 8.5; NaCl 7.5%) MRS broth was inoculated with *Lb. plantarum* and incubated at 30°C (except for stress conditions of temperature: 10°C, 42°C). Changes in pH and optical density were recorded in every hours for 48h. Bacteriocin activity in the cell-free supernatant was recorded in every 3 hours for 48h. *Listeria monocytogenes* serogroup IIb (from cheese), *L. monocytogenes* serogroup IVb (from cheese), *L. monocytogenes* serogroup IIb (from hamburger) and *L. innocua* NCTC 11288 were used as target strains.

Results: *Lactobacillus plantarum* could not grow well under osmotic stress but it was able to produce low amount of bacteriocin. At pH 8.5 the alkaline adaption was clearly observed and it took ~ 20 hours. For pH 3.5 and high temperature *Lb. plantarum* was able to grow and produced bacteriocin. At low temperature LAB could grow but was not able to produce bacteriocin.

Significance: It was demonstrated that under stress conditions (except low temperature), *Lb. plantarum* could produce antilisterial bacteriocins. *L. monocytogenes* IIb was the most sensitive to the tested bacteriocins, while *L. innocua* showed to be more resistant.

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T11-05 A Novel Antimicrobial Sanitizer for Enhancing Microbial Safety of Whole Melons Designated for Fresh-cut Preparation

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Introduction: Wash-water chlorinated up to 200 ppm is routinely applied to reduce microbial contamination in produce processing lines. However, the use of chlorine is of concern due to the potential formation of harmful by-products. Therefore safer alternative sanitizers for fruits and vegetables are needed.

Purpose: Lovit, a sanitizer that consists of short chain organic acids generally regarded as safe (GRAS) in combination with nisin + ethylenediaminetetraacetic acid (EDTA) was developed and used to investigate the survival and inactivation of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* bacteria on produce surfaces (cantaloupe, watermelon and honeydew).

Methods: *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* were inoculated on cantaloupe, watermelon and honeydew rind surfaces at 4.2 ± 0.21, 4.8 ± 0.14 and 3.9 ± 0.17 log CFU/cm^2, respectively. Inoculated produce were washed with water, hydrogen peroxide (3 %), chlorine (200 ppm) and Lovit solutions for 5 min. Bacterial populations that survived treatments and transferred to fresh-cut pieces were estimated using selective agar media.

Results: The Lovit treatment led to viability loss of 3.5 log for three pathogens tested on cantaloupe and a loss of 2.8 log on watermelon and honeydew rind surfaces, compared to 2.2 log reduction by hydrogen peroxide and chlorine. In fresh-cut cantaloupe pieces prepared from Lovit treated melons, the populations of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* bacteria were below detection (< 1 CFU/g) and honeydew and watermelon fresh-cut pieces were negative for these pathogens by enrichment. An average of 0.74 ± 0.12 log CFU/g of all three pathogens were recovered from fresh-cut pieces prepared from chlorine and peroxide treated whole melons.

Significance: Treating whole melon surfaces with Lovit solution before fresh-cut preparation will improve the microbial safety and quality of the prepared fresh-cut pieces, and will drastically reduce the incidence of foodborne illness and costly recalls of contaminated produce.

T11-06 Resistance of Human Norovirus to Common Sanitizers

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Introduction: Previously the porcine gastric mucin-magnetic bead (PGM-MB) assay was shown to discriminate between infectious and inactive human norovirus (HuNoV; Dancho et al., 2012).

Purpose: Here, the PGM-MB assay was used to evaluate the ability of common sanitizers to inactivate human Norovirus (HuNoV) within 10% stool filtrate.

Methods: Treatments for one min with free chlorine at concentrations of 33 and 189 ppm, for five min with 5% trisodium phosphate (pH 12), for up to 1h with 350 ppm chlorine dioxide dissolved in water, for 1 min treatments with up to 195 ppm PAA, and for up to 60 min with hydrogen peroxide (4%) were performed followed by the PGM-MB binding assay.

Results: Chlorine treatments reduced virus binding in the PGM-MB assay by 1.48 and 4.14 log, respectively, suggesting that chlorine is an efficient sanitizer for HuNoV. Trisodium phosphate (pH 12) reduced HuNoV binding by 1.6 log, suggesting that TSP, or some other high pH buffer, could be used to treat food and food contact surfaces to reduce HuNoV. One min treatments with 350 ppm chlorine dioxide dissolved in water did not reduce PGM-MB binding, suggesting that the sanitizer may not be suitable for HuNoV inactivation in liquid form. However a 60 min treatment with 350 ppm chlorine dioxide did reduce human norovirus by 2.8 log, indicating that chlorine dioxide had some activity against HuNoV. Peroxyacetic acid (PAA) had limited effectiveness against HuNoV, since HuNoV binding was reduced by less than 1 log. Hydrogen peroxide treatment resulted in minimal binding reduction (0.1 log) suggesting that hydrogen peroxide is not a good liquid sanitizer for HuNoV.

Significance: Overall this study suggests that HuNoV is remarkably resistant to several commonly used disinfectants and advocates for the use of chlorine (sodium hypochlorite) as a HuNoV disinfectant wherever possible.

T11-07 Rapid Destruction of Human Norovirus Capsid and Genome Occurs during Exposure to Copper-containing Surfaces

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Developing Scientist Competition

Introduction: Human norovirus (HuNoV) infection represents a significant public health burden. Infectious virus particles can persist on surfaces for weeks and can serve as sources of contamination to people and foods. Unfortunately, HuNoV are resistant to many common disinfectants. Copper surfaces have been shown to inactivate the cultivable surrogate murine norovirus, but no such data exist for HuNoV.

Purpose: To characterize the destruction of HuNoV after exposure to five copper alloy surfaces (ranging from 60-99.9% copper).

Methods: Diluted stool positive for GI.6 or GII.4 HuNoV or GII.4 virus-like particles (VLPs) were placed onto copper or stainless steel (control) surfaces and recovered by elution at various time points (0 to 240 minutes). To assess HuNoV genome integrity after exposure, viral RNA was extracted and analyzed by RT-qPCR. To assess capsid integrity, exposed VLPs were visualized by transmission electron microscopy (TEM) and also analyzed by SDS-PAGE/Western blot. Additionally, a histo-blood group antigen (HBGA) binding assay was used to evaluate VLP receptor binding ability after exposure to copper surfaces.

Results: Exposure to copper surfaces resulted in higher reduction of HuNoV genome copy number compared to stainless steel surfaces ($P < 0.05$). Pure copper surfaces reduced HuNoV genome copy number by 4-log RT-qPCR units within 30-60 minutes. As visualized by TEM, exposure to copper (but not stainless steel) resulted in VLP clumping and destruction within 120 and 240 minutes, respectively. Exposure to copper surfaces resulted in significant reduction in protein band intensity by SDS-PAGE/Western blot ($P < 0.05$) and reduced the ability of VLPs to bind to their HBGA receptor ($P < 0.05$).

Significance: The results demonstrate that exposure to copper alloy surfaces results in the destruction of the genome and capsid of HuNoV. These surfaces may have utility in preventing spread of HuNoV in settings such as restaurants, schools, and hospitals.

T11-08 Meta-analysis of the Published Literature on the Effectiveness of Hand Sanitizers

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Developing Scientist Competition

Introduction: The U.S. FDA model food code currently allows hand sanitizers to be used following proper handwashing in foodservice establishments. In contrast, the U.S. CDC has recommended alcohol-based hand sanitizers as an alternative for hand washing in healthcare settings.

Purpose: Meta-analyses contrast and combine results from different studies to identify patterns, sources of disagreement, or other interesting relationships that may come to light in the context of multiple studies. This study was undertaken in an effort to determine those factors contributing to hand sanitizer effectiveness.

Methods: A search of hand sanitizer literature was conducted, and included searching for published data on the effects of hand sanitizers on bacteria and viruses. Data on sample size, experiment protocol used, sanitizing agent, concentration of antimicrobial, exposure time, exposure volume, organism, mean starting microbial concentration, and log reduction were recorded and a database was complied. Histograms, linear regression analysis, ANOVA, and post hoc Tukey's range test were all used to analyze the data.

Results: Twenty-eight publications, containing 336 observations, met the criteria for the study. There was a significant difference ($P < 0.001$) between ethanol (EtOH) and isopropanol (IsoP) effectiveness. IsoP had a higher mean log reduction (4.2 log CFU) than EtOH (3.7) for bacterial data sets, but not for virus datasets. Significant differences were seen between testing protocols. Log reductions, as measured by fingerpad and glove juice methods (both 1.5 mean log reduction), were significantly lower than those based on European Standard EN 1500 methods (3.5 mean log reduction). Alcohol-based hand sanitizers (3.8 mean log reduction) were more effective ($P < 0.005$) than those based on other antimicrobials (2.6 mean log reduction).

Significance: The choice of a testing protocol has a great influence on measured hand sanitizer effectiveness. Alcohol-based hand sanitizers are more effective than those based on other antimicrobials for both bacteria and viruses.

T11-09 Metal Chelating Active Packaging Film Enhances Activity of Food Grade Antimicrobials

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Developing Scientist Competition

Introduction: Several studies have demonstrated that metal chelators enhance the antimicrobial activity of lysozyme. There has yet to be any investigation of the effect of non-migratory metal chelating active packaging films on the antimicrobial activity of lysozyme.

Purpose: The objective of this study was to characterize the antimicrobial synergy between lysozyme and metal chelating active packaging.

Methods: Polypropylene films were surface modified by photoinitiated graft polymerization of acrylic acid (PP-*g*-PAA) onto the food contact surface of the films to impart chelating activity based on charge interactions. To quantify synergistic antimicrobial activity between lysozyme and the chelating active packaging film, a microtiter assay was developed using a 96 well microarray microtiter plate, in which swatches of film are assembled at the bottom of the plate and function as the bottom of each well.

Results: PP-*g*-PAA exhibited a carboxylic acid density of 113 ± 5.4 nmol cm^{-2} and an iron chelating activity of 53.7 ± 9.8 nmol cm^{-2}. The antimicrobial interaction of lysozyme and PP-*g*-PAA against *Listeria monocytogenes* depended on the media composition. PP-*g*-PAA decreased lysozyme activity at low ionic strength (2.48 log increase at 64.4mM total ionic strength) and enhanced lysozyme activity at moderate ionic strength (5.22 log reduction at 120mM total ionic strength). These results support the hypothesis that, at neutral pH, synergy between carboxylate metal chelating films (pKa^{bulk} 6.45) and lysozyme (pI 11.35) is optimal in solutions of moderate ionic strength. These findings suggest that chelating active packaging with high metal affinity based on ligand-specific interactions, not charge-based interactions, may enable enhanced synergy with membrane-disrupting antimicrobials across a wider range of pH values and ionic strengths.

Significance: This work demonstrates the potential application of metal chelating active packaging films to enhance activity of food grade antimicrobials.

T11-10 Development of Less Pungent Antimicrobial Coatings and Films Containing Essential Oil

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Introduction: Essential oils have been intensively studied as antimicrobials against pathogens and spoilage microorganisms in foods. However, due to high volatility and strong odor, their applications are limited. Therefore, research is needed to develop new methods to incorporate essential oils into edible coating solutions or films that have stable antimicrobial activity without detectable odor.

Purpose: The objective of this study was to develop new edible antimicrobial coating solutions and films using natural biopolymers and high pressure homogenization technology.

Methods: Allyl isothiocyanate (AIT, 5%), barley fiber gum (BFG, 0.5%) and chitosan (3%) were used as antimicrobial, emulsifier, and film former, respectively, and were mixed in an acid solution (2%). The mixture was treated by high pressure homogenization (HPH, 138 MPa, 3 cycles). The solution was vacuum dried to form an edible packaging film. Antimicrobial activities of the solution used alone or the films against *Listeria innocua* in tryptic soy broth (TSB) or on deli meat were investigated. Physical properties including transparency, color, and binding capacity on other plastic films were also evaluated. The release of AIT from solutions/films was determined by GC-MS method.

Results: Films with BFG were softer, less rigid and more stretchable than those without BFG. HPH treatment significantly ($P < 0.05$) reduced the particle sizes in the emulsion to less than 1 µm. Both BFG and HPH significantly reduced the pungent odor of AIT in films without losing its antimicrobial activity and enhanced the binding capability of the solutions on the plastic films. The films reduced *L. innocua* by up to 5 log CFU in TSB, and also significantly inhibited its growth on meat surface.

Significance: The developed method can be used for other pungent essential oils. This study provides an innovative approach to develop edible antimicrobial coating solutions and films to reduce potential microbial contaminations on foods, enhance food safety, and extend shelf life.

T11-11 Phenotypic and Genotypic Toxin and Antimicrobial Resistance Traits of *Staphylococcus aureus* Isolates from China

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Introduction: *Staphylococcus aureus* can produce a wide variety of virulence factors. As major virulence factors in *S. aureus*, toxic shock syndrome toxin (TSST), exfoliative toxins (ETs), and enterotoxins (SEs) have been implicated in host colonization, invasion of damaged skin and mucus, gastrointestinal infection, and evasion of host defense mechanisms. Meanwhile, the transmission of antibiotic resistant *S. aureus* (ARSA), especially methicillin-resistant *S. aureus* (MRSA), between food products and humans has become a serious problem.

Purpose: The aim of this study is to determine the toxin gene profiles of *S. aureus* isolates from different clinic and food samples in order to understand the genetic and pathogenic relatedness, as well as its epidemiology, as well as to monitor the antimicrobial resistance profiles of this bacterium through the food supply chain.

Methods: In this study, a total of 108 *S. aureus* isolates from 16 major hospitals located in 14 different provinces in China were characterized for the profiles of 18 staphylococcal enterotoxin (SE) genes, 3 exfoliatin genes (*eta, etb* and *etd*), and the toxic shock syndrome toxin gene (*tsst*) by PCR. The genomic diversity of each isolate was also evaluated by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and accessory gene regulator (*agr*) typing. Furthermore, the disk diffusion method and E-test were performed to determine the prevalence of ARSA in 135 foodborne *S. aureus* isolates using 18 antibiotics. PCR screening for the presence of 14 genes conferring antibiotic resistance was conducted.

Results: Of these clinic isolates, 90.7% (98/108) harbored toxin genes, in which *tsst* was the most prevalent toxin gene (48.1%), followed by *sea* (44.4%), *sek* (42.6%) and *seq* (40.7%). The *see* and *etb* genes were not found in any of the isolates tested. Because of high-frequency transfer of toxin genes contained in mobile genetic elements among *S. aureus* isolates, a total of 47 different toxin gene combinations were detected, including a complete *egc* cluster in 19 isolates, co-occurrence of *sea*, *sek* and *seq* in 38 isolates, and *sec* and *sel* together in 11 isolates. Genetic typing by PFGE grouped all the isolates into 25 clusters based on 80% similarity. MLST revealed 25 sequence types (ST) which were assigned into 16 clonal complexes (CCs) including 2 new singletons. Among these, 11 new and 6 known STs were first reported in the *S. aureus* isolates from China. Overall, the genotyping results showed high genetic diversity of the isolates regardless of their geographical distributions, and no strong correlation between genetic background and toxin genotypes of the isolates. For genotyping *S. aureus*, PFGE appears to be more discriminatory than MLST. However, toxin gene typing combined with PFGE or MLST could increase the discriminatory power of genotyping *S. aureus* isolates. Among the antimicrobial resistance profiles of 135 foodborne isolates, the highest resistance frequency was found for penicillin G (74.8%), followed by erythromycin (52.6%)

and ciprofloxacin (32.6%), whereas no vancomycin-resistant isolates were found. Eight MRSA isolates were found in this study. These isolates could be subtyped into 62 resistance profiles and 26 clusters based on their antimicrobial susceptibility. The presence of resistance genes was relatively high: bla_{TEM} (78.5%), *ermB* (38.5%), *ermC* (37.8%) and *aac6'/aph2"* (36.3%). The incidence of antibiotic resistance was significantly correlated to food types (P = 0.018), with isolates from meat and raw milk more resistant to antibiotics than those from frozen food and vegetables.

Significance: The toxin and antimicrobial resistance of *S. aureus* have become a serious concern in clinics and foodstuffs. The results of this study will assist the better understanding of the toxin and antimicrobial resistance profiles of those isolates from different sources. The appropriate genotyping method for the *S. aureus* isolates has also been recommended.

T11-12 Antimicrobial Genotyping of *Salmonella* Isolates with a Comparison of Serotype and Source (Food, Animal and Human) Distribution

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Developing Scientist Competition

Introduction: Salmonellosis, one of the most common and widely distributed foodborne diseases, may cause severe health problems depending on host factors (i.e., infants, elderly and immunocompromised patients) and strain of *Salmonella.* Antimicrobial therapy is necessary for the health risk groups. On the other hand, there is a global increase of antimicrobial resistance due to redundant and unconscious usage. Multidrug-resistant (MDR) *Salmonella* is a great concern to health in severe salmonellosis cases, when this multidrug resistance interferes with treatment.

Purpose: The objective of this study is to determine the genotypic variations of the antimicrobial resistance profile among *Salmonella* isolates from farm/field to fork (i.e., animal, food and human) collected in Turkey, 2012.

Methods: Strains were gathered from southeast and median Anatolian region of Turkey. The isolates were from veterinary, human and food. For *Salmonella* isolation, ISO6579 procedure was used. The confirmation of *Salmonella* isolates was done by *invA* gene on PCR. Serotyping of *Salmonella* was performed according to the Kauffman-White Procedure. Phenotypic antimicrobial resistance typing was done by disk diffusion method. 19 different antimicrobial elements were used. 21 antimicrobial resistance genes were searched in 90 phenotypically resistant *Salmonella* isolates.

Results: 74 food-related, 54 animal-related and 50 clinical human *Salmonella* isolates were investigated. 26 different serotypes were determined. Chicken isolates take the attention since all were found to be resistant at least one antimicrobial agent, and most of them belong to serotype *S.* Infantis (90.5%). Every Infantis isolate was resistant to nalidixic acid and tetracycline. Differently from food isolates, animal isolates were observed to have high rate of resistance against beta-lactam groups. Antimicrobial resistance profiles of *Salmonella* Hadar isolates that were obtained from food and animal samples were similar.

Significance: Our study fills the gap of limited relevant studies about the antibiotic susceptibility profile of *Salmonella* isolates from farm/field to fork in Turkey. Our study has the potential of being a progressive work conducted in the pathogenicity area.

Poster Abstracts

P1-01 Evaluation of Commercial Test Kits for Detection of *Escherichia coli* O157:H7 in Alfalfa Sprout Spent Irrigation Water

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Introduction: Microbiological testing of spent irrigation water has been recommended as part of an overall strategy to reduce sprout-associated outbreaks. An increasing number of pathogen screening kits based on different assay formats are commercially available and many of them have been certified by the AOAC. However, very few have been validated for detection in sprouts or spent irrigation water.

Purpose: Compare the performance of AOAC certified rapid methods with the FDA BAM method for detection of *Escherichia coli* O157:H7 in artificially inoculated alfalfa sprout spent irrigation water. The methods selected include four lateral flow tests (Reveal, RapidChek, Singlepath, VIP Gold), three enzyme immunoassays (VIDAS UP, Assurance EIA, TECRA VIA), and six DNA-based assays (BAX MP, GeneDisc, iQ-Check, MicroSEQ, foodproof, Assurance GDS).

Methods: Twenty-five ml of spent irrigation water collected from a commercial sprouting facility was inoculated with 0, 1, or 10 CFU of *E. coli* O157:H7 and was subjected to the recommended enrichment and assay protocols, including both the shorter version (6-10h) and the 16-24h enrichment. Regardless of assay results, the presence of *E. coli* O157:H7 in the enrichments was determined using procedures outlined in the FDA BAM.

Results: Following the 16-24h enrichment protocols, all the DNA-based methods were able to detect the presence of *E. coli* O57:H7 at the 10 CFU/25 ml level, although performances differed when testing samples spiked at the 1 CFU/25 ml level. Assurance GDS and BAX performed better, probably due to the use of more selective enrichment media. For the immunoassay-based methods, using the shorter enrichment protocols, all methods underperformed when compared with the BAM, with VIP Gold and Assurance EIA performing better than others. When the longer enrichment protocols were followed, Reveal, RapidChek, VIDAS UP, and Tecra performed equally or better than the BAM.

Significance: Not all AOAC certified methods can perform well for testing low levels of *E. coli* O157:H7 in sprout spent irrigation water. Enrichment media used by each test kit play a key role in determining the sensitivity of the method.

P1-02 Evaluation of Novel Phage Protein Derived Latex Agglutination Assays for the Confirmation of the Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups

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Introduction: Latex agglutination is a part of the diagnostic methodologies including USDA-MLG for the confirmatory identification of STEC. Slidex *Escherichia coli* serogroup assays are novel latex agglutination assays incorporating latex beads coated with recombinant bacteriophage proteins highly specific for STEC serogroups including O157, O145, O26, O111, O121 and O103.

Purpose: Evaluation of the new latex agglutination assays for the identification of STEC colonies on selective chromogenic medium from pure cultures and artificially inoculated beef samples.

Methods: A panel of 110 strains of target STEC serogroups and 40 non-target strains isolated on selective chromogenic (chromID EHEC) and non-selective (TSAB) media were tested using the latex assays to study inclusivity, exclusivity, and time to agglutination. A portion of colonies were tested with negative controls to rule out any autoagglutination. For the inoculation study, 325-g samples of raw beef were individually spiked with 25-100 CFU of 30 STEC strains (five per serogroup) and following enrichment and immunoconcentration, colonies were isolated on the chromogenic medium. Up to five typical colonies were analyzed using the latex assays and positive colonies were confirmed using the serogroup-specific PCR. Uninoculated samples were confirmed negative before the experiments.

Results: The latex assays showed 100% inclusivity with all 110 STEC strains resulting in a distinguished visible agglutination within 10-20 sec from both media. None of the 40 non-target strains showed any cross-reactivity with the latex. In the inoculated beef samples study, characteristic colonies isolated on chromogenic medium from each sample resulting in a positive agglutination reaction were confirmed to target STEC using serogroup specific PCR. The blue colored latex made it easy to read and interpret the agglutination reaction.

Significance: Due to high level of specificity, recombinant phage protein based latex assays eliminate the possibility of cross-reactivity with non-target isolates. Further, the highly sensitive latex assays provide quick and easy-to-read high intensity agglutination reactions with target antigens.

P1-03 Use of Specific CRISPR-2 Spacers for the Detection of the Big Six STEC Serotypes by Real-time PCR

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Introduction: Molecular typing of pathogenic *Escherichia coli* is important for diagnostic microbiology and trace back epidemiology to quickly establish food-clinical linkages and as an indicator of virulence potential. A clustered, regularly interspaced, short palindromic repeat (CRISPR) presents an alternative genetic marker for molecular typing and detection of pathogenic Shiga toxin-producing *E. coli* (STEC).These regions are conserved among phylogenetically related *E. coli,* and can be used as genomic signatures for food safety related applications.

Purpose: The purpose of this study was to evaluate the application and utility of CRISPR loci and spacers as potential genetic markers in molecular sub-typing of several STEC strains including the "big six" serotypes O145, O121, O103, O111, O26 and O45 by a real-time PCR assay.

Methods: One hundred different *E. coli* strains from our collection were tested. The CRISPR-2 regions and spacers of the big six *E. coli* serotypes were identified and 30 conserved spacers were selected based on BLAST information and http://crispr.u-psud.fr/CRISPRcompar. Real-Time PCR assays were performed in a volume of 25 µl in 96-well plates using the 30 spacer panel.

Results: We have analyzed over 100 strains using the panels and no template control (NTC) was used as a negative control. We were able to distinguish the big six serotypes using the cycle threshold (Ct) value of 30; the Ct value < 30 was considered positive.

Significance: Our data demonstrated CRISPR-2 spacers could be utilized as molecular markers associated with the big six serotypes with proper databasing to reference strains associated particularly with each *E. coli* serotype. Further studies include expansion of spacer repertoire for broader application to *E. coli* serotypes beyond the "big six."

P1-04 Evaluation of NanoLuc® and GFP Reporter-labeled Control Strains for Shiga Toxin-producing *Escherichia coli* (STEC) Assays

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Introduction: Under the FDA Food Safety Modernization Act and the USDA-FSIS requirements, positive controls are essential for the detection of STEC in non-intact raw beef samples. In order to effectively discriminate between positives controls and actual food adulterant contamination, strains should contain unique and easily detectable reporters.

Purpose: To evaluate QC strains labeled with NanoLuc® Luciferase (Promega) and GFP reporters as positive controls for use in food testing following criteria outlined in the USDA-FSIS protocol for *Escherichia coli* O157:H7.

Methods: Plasmids bearing either NanoLuc® or GFP reporters were transformed into 8 STEC strains: Big-Six *E. coli* (ATCC® MP-9™) and *E. coli* O157:H7 toxigenic (ATCC® 35150™) and non-toxigenic (ATCC®43888™) strains. The stability of the reporters was determined through unselective serial passage. The growth rate, chromogenic properties, and molecular profile of reporter-labeled and native strains were compared.

Results: The signal strength of both reporters was readily detectable by the visual inspection of cells grown in broth culture and on agar plates. Following unselective serial passage, the reporters could be detected in ≥70-100% of colonies for ≥ 2 days. Doubling times between reporter-labeled strains and native strains were tested in triplicate and varied between 0% and 39.4%, in a strain-specific manner. No changes were observed in the chromogenic properties of the reporter-labeled or native strains grown on Rainbow Agar™. Further, the PCR profiles of the *stx1*, *stx2* and *eaeA* genes were unchanged between the reporter-labeled and native strains.

Significance: This study demonstrates that the NanoLuc® and GFP reporter labeled QC strains can be routinely used as positive controls for STEC detection assays.

P1-05 Comparison of chromID EHEC, Modified Rainbow Agar, and Rainbow Agar for the Isolation and Confirmation of USDA Top 7 Shigatoxigenic *Escherichia coli* from Pure Culture and Beef Trim Enrichment Using FSIS MLG 5.06 and MLG 5B.03 Methods

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in several foodborne outbreaks. It can be difficult to find the target STEC with the chromogenic media used by the MLG method.

Purpose: The purpose of this study was to evaluate a new culture media, chromID EHEC (c-EHEC), for the detection of the USDA top 7 STEC.

Methods: Isolates of each of the 7 STEC were tested using pure cultures and inoculated beef trim. For pure cultures, organisms were incubated according to MLG 5B.03. Two cultures from each serogroup were streaked on c-EHEC and MLG described media. The remaining cultures were diluted, concentrated and plated to the same media. For the matrix study, 25g of beef trim was inoculated then enriched per MLG 5B.03. The enrichments were concentrated and plated to c-EHEC media and either mRBA or RBA media per MLG methods. After incubation, suspect colonies were tested with O specific latex. Presumptive positive colonies were tested by PCR or H7 latex after incubation on blood agar.

Results: For pure cultures, one isolate taken through the IMS process and one organism streaked directly to plates did not grow on either media because of mTSB + novobiocin sensitivity. All other organisms grew well on both media. For the matrix study, neither media was able to grow all target organisms. The target organisms had less color variance on the c-EHEC media compared to the mRBA or RBA plates. When a colony from c-EHEC media was latex-positive, it was also PCR-positive more often than when colonies from RBA media were latex-positive.

Significance: The c-EHEC media worked as well as the mRBA or RBA media for the detection of the 7 STEC from pure culture and from inoculated beef trim and provides a potential alternative to augment or replace the mRBA media described in the MLG 5B.03 method.

P1-06 Rapid Detection of Non-O157 STEC *Eschericha coli* Using a Flow Cytometry-based Pathogen Detection System

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Introduction: Beef producers need a reliable rapid technology for *Escherichia coli* STEC testing. Current rapid technologies require several hours of enrichment and additional time on the rapid technology instrument. A flow cytometry-based (FCB) system improves the turn-around for results with a quicker enrichment requiring only 6.5 hours enrichment for a 25-gram sample and 8.5 hours enrichment for a 375-gram sample, with only an additional 30 minute time to result after enrichment.

Purpose: The purpose of this study was to validate the FCB system for non-O157 STEC testing compared to the FSIS/USDA reference method.

Methods: A total of 10 samples per non-O157 STEC strain (O26, O45, O103, O121, O111 and O145) were inoculated (5 ground samples and 5 trim samples) using a batch method. Three sample groups were tested, a 25-gram sample using the FBC system, a 375-gram sample using the FCB system, and a 325-gram sample using FSIS/USDA reference method. All samples were enriched and tested according to the methods standard operating procedure. Every FBC sample was additionally culturally confirmed using the FSIS/USDA reference method.

Results: A total of 60 ground samples and 60 trim samples were inoculated and tested using the FBC technology. For the ground samples, 59 were positive on the FCB system. All 59 also culturally confirmed out, and the one negative sample was negative by cultural confirmation. For the trim samples, 40 were positive on the FBC system. All 40 also culturally confirmed out, and the 20 negative samples were also negative by cultural confirmation.

Significance: The results of the study show both sample types (ground and trim) had 100% sensitivity and 100% specificity using the FBC technology. Based on this study, the FBC system (Rapid-B Pathogen Detection System, Vivione Biosciences) offers beef producers a faster method for the rapid detection of non-O157 STEC testing.

P1-07 Evaluation of Commercial Agar for the Detection of Shiga Toxin-producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) of serogroups O26, O45, O103, O111, O121, O145, and O157 have been declared adulterants in non-intact, raw beef by the USDA-FSIS, and enteroaggregative STEC O104:H4 is an additional concern.

Purpose: The objective of this study was to compare the sensitivity, specificity, and accuracy of CHROMagar™ STEC (CS) and R&F®Non-O157 STEC chromogenic plating medium (RF) for detection of STEC using pure cultures.

Methods: A total of 79 STEC (divided among O26, O45, O103, O104, O111, O121, O145, and O157) and 38 non-STEC strains were included in the study. Strains were grown in trypticase soy broth, then streaked onto agar media using a semi-quantitative loop technique. After 22h incubation, plates were evaluated for growth (scored from 0-4), and colony phenotype and compared to the expected results as provided by the manufacturer. Growth on these media was also compared to that on trypticase soy agar (TSA).

Results: The sensitivity of RF (95.0%; 95% confidence interval [CI], 89.9 to 98.0%) was significantly greater than that of CS (82.4%; CI, 75.9 to 77.7%; $P < 0.05$), whereas the specificities of CS (80.7%; CI, 70.9 to 88.8%) and RF (76.8%; CI, 66.2 to 85.4%) were not significantly different. The accuracy of RF (88.2%; CI, 83.2 to 92.2%) was marginally higher than that of CS (81.8%; CI, 76.6 to 86.3%; $P = 0.06$). Growth of STEC on RF (3.26) was significantly greater ($P < 0.05$) than that on CS (2.60), as was growth of non-STEC controls (2.05 versus 0.81). Growth of non-STEC controls (3.49) on TSA was significantly higher than on the other media.

Significance: R&F® Non-O157 STEC chromogenic plating medium yielded the best combination of sensitivity, specificity and accuracy for detection of non-O157 STEC. However, further studies are needed to assess performance in different food and environmental matrices, as these could significantly influence results.

P1-08 Evaluation of Enrichment and Transport Media for Detection and Enumeration of Shiga Toxin-producing *Escherichia coli*

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Introduction: Food industry, research and regulatory interests in Shiga toxin-producing *Escherichia coli* (STEC) continue to increase, placing emphasis on optimization of cultural protocols that ensure detection (when target serotypes are present). Also important but often overlooked is the stability of STEC population levels in samples collected in the field and shipped to a laboratory for analysis, particularly in research settings where quantification of targets is an objective. Much of the current cultural methodology currently applied to STEC analyses was developed for serotype O157, and growth/survival characteristics of other serotypes is not well established.

Purpose: This experiment was designed to characterize growth of seven beef adulterant STEC serotypes (STEC-7) in different media types for sample enrichment, and compare population level stability of STEC-7 in common transport media during short-term storage at refrigerated and slightly abusive temperatures sometimes encountered during sample shipment.

Methods: STEC-7 were individually inoculated into enrichment media (EC broth, Buffered Peptone Water, Universal Pre-enrichment Medium, and Tryptic Soy Broth; with and without novobiocin supplementation) and growth curves at 37°C were generated and compared. STEC-7 were inoculated into three transport media (Cary-Blair, Maximum Recovery, and Buffered Peptone Water), stored at 4 and 10°C, and sampled over 72h to compare stability in relation to inoculation concentration.

Results: EC broth supported rapid growth of all STEC-7 and was similar to non-selective media. Media containing novobiocin greatly restricted growth of most non-O157 serotypes. Cary-Blair transport medium maintained population level stability during storage at 10°C while other media allowed increases. All transport media was acceptable at 4°C storage.

Significance: Following common *E. coli* O157:H7 enrichment protocols to detect STEC-7 may lead to false-negative determinations, particularly if novobiocin is included as a selective agent. When transporting field samples to a laboratory, Cary-Blair Medium should be considered to protect STEC-7 in the event that temperature abuse of samples occurs.

P1-09 Detection and Isolation of Shiga Toxin-producing *Escherichia coli* (STEC) O104 and Other STEC Serogroups of Public Health Concern

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens that cause outbreaks and serious cases of foodborne illness. Methodologies for detection and isolation of STEC, particularly the non-O157 STEC, are needed to prevent their transmission through contaminated food.

Purpose: The objectives of this study were to develop antibodies against different STEC serogroups and produce immunomagnetic separation (IMS) and latex reagents. Furthermore, methodologies were developed to detect and isolate STEC O104 and enteroaggregative STEC (EAEC-STEC) O104:H4 in sprouts, utilizing multiplex PCR assays and the IMS and latex reagents.

Methods: Antibodies against the top six non-O157 STEC serogroups (O26, O45, O103, O111, O21, and O145), as well as STEC O104 and O157 were generated in rabbits and used to produce IMS and latex beads for the different pathogens. Specificity testing was performed using the target pathogens and non-target *E. coli*. A method was developed for detection of STEC O104 and EAEC-STEC O104:H4 in sprouts, consisting of a selective enrichment, two real-time multiplex PCR assays (stx_2, *aggR*, and wzx_{104} genes for detection of EAEC-STEC O104:H4 and $stx_{1\text{-}2}$, *ehxA*, and wzx_{104} for STEC O104), followed by IMS, isolation from selective agars, and confirmation by latex agglutination and PCR.

Results: The IMS and latex reagents against all of the STEC serogroups showed good specificity and were useful for isolating the target pathogens, as shown by plating onto selective and non-selective agars. Cold stressed STEC and EAEC-STEC O104 were detected and isolated from sprout samples inoculated at a level of less than 1 CFU/g. All presumptive colonies were confirmed by agglutination using the O104 latex particles and the multiplex PCR assays.

Significance: The IMS and latex reagents and the methods developed in this study improve the ability of the food industry and regulatory agencies worldwide to detect and isolate important STEC from food.

P1-10 Antibiotic Resistance of *Escherichia coli* Strains Isolated from Avian Samples in the Region of Algiers (Algeria)

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Introduction: *Escherichia coli* is currently in poultry one of the most important causes of economic losses, it induces diseases and constitute one of the most frequent motives for seizure in the slaughterhouse. The antibiotics resistance of bacteria constitutes a real threat for the animal health and the human sanitary safety. The emergence of resistant strains would be the result of improper and permanently use of the different families of antibiotics in animals, in particular as growth promoters.

Purpose: This work aims at the study of the antibiotic susceptibility of *E. coli* strains isolated from various samples of avian origin in the northern region of the country. The total number of isolated strains increased from 276 in 2004 to 822 in 2008.

Methods: This study includes the data of the seven regional veterinary laboratories of the National Institute of Veterinary Medicine from 2004 to 2008, by using the reference technique recommended by the OIE for the strains isolation, and the CLSI method for the achievement of antibiograms.

Results: The rates of resistances recorded for the most part of tested antibiotics are alarming. For the ampicillin this rate passed from 63% in 2004 to 83% in 2008. For the neomycin, the cotrimoxazole, the enrofloxacine, the gentamicin, the oxytetracycline, the furanes and the chloramphenicol this rate achieved in 2008, respective values of 45%, 68%, 63%, 8%, 82%, 23% and 26%.

Significance: High rates of resistance were recorded, which represents a potential danger to public health.

P1-11 Differentiation of Colony Morphology of Shiga Toxin-producing *Escherichia coli* on Commercial Agar Media

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Introduction: Shiga toxin-producing *Escherichia coli* (STECs) which include O157, O26, O45, O103, O111, O121, and O145 can cause serious illness in humans. Hence, the USDA-FSIS has announced a zero-tolerance policy for these seven serotypes in raw beef products. Because of the close relatedness among these serotypes, the ability to specifically differentiate their colonies on an agar culture medium is extremely difficult.

Purpose: The purpose of this study was to compare among three commercial agar media, chromID® EHEC agar, EZ-CHROM STEC agar and Rainbow® agar, in their ability to effectively differentiate among colonies of these seven STEC strains.

Methods: Pure overnight culture of each STEC serotype was streaked onto chromID® EHEC agar, EZ-CHROM STEC agar and Rainbow® agar**.** Combinations of each strain, up to a multiple of six strains, were also streaked and colony differentiation was noted on each agar medium. Final confirmation of each well isolated colony of each serotype was performed using serotype-specific primers and real-time PCR.

Results: EZ-CHROM STEC generated similarly colored colonies for most serotypes which were hard to differentiate and, hence, was discontinued from the study. chromID® EHEC agar was able to clearly differentiate a cocktail of up to five serotypes, while the color differentiation obtained for the five serotype mixture was not distinguishable on Rainbow® agar. PCR results for the colonies picked from mixed culture chromID® EHEC agar plates were in accordance with the morphological characteristics of each serotype, except for *E. coli* O145 and *E. coli* O45.

Significance: Out of the three chromogenic media tested, chromID® EHEC agar produced the most distinctive and easily distinguishable colored STEC colonies and was the most effective for the screening and identification of the STEC serotypes in this study.

P1-12 Multiplex Real-time PCR Assay for Detection of Eight STEC Serotypes

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Developing Scientist Competition

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are strains of *E. coli* that cause serious illness, including fatal hemolytic uremic syndrome. This group consists of STEC serotypes O157, O26, O45, O103, O111, O121 and O145 that are generally associated with raw meat and are also responsible for more than 71% of infections. Thus, in addition to STEC O157, the USDA FSIS also declared these six serotypes as adulterants.

Purpose: The aim of this study was to design a multiplex real-time PCR assay for detection of eight STEC serotypes.

Methods: Serotype-specific PCR primers were designed for the specific amplification of each STEC serotype. Final primer sets were selected based on amplicon T_m, reaction efficiency, and amplicon size. Two multiplex melt curve real-time PCR assays with an internal amplification control (IAC) were standardized for the detection of eight STEC serotypes. The applicability of the assays was tested using 11 different meat and produce samples.

Results: The first multiplex assay detected *E. coli* O145, *E. coli* O121, *E. coli* O104, *E. coli* O157 and IAC; while the second set targeted *E. coli* O26, *E. coli* O45, *E. coli* O103, *E. coli* O111 and IAC. Following an enrichment period of 6h, all targets of the multiplex assays could be detected in food samples contaminated with a cocktail of four STEC serotypes with a combined count of 10 CFU/25 g food. The assay showed a highly reproducible result for nine food samples tested in this study.

Significance: The assay developed in this study can be used for the detection of eight STEC serotypes and can be completed in less than 11h. Unlike other commercially available methods, it does not require fluorescent-labeled probes or immunomagnetic beads, making it one of the shortest and most commercially feasible methods available.

P1-13 MALDI-TOF MS-based Approach for Discrimination of Enterohemorrhagic *Escherichia coli* O157, O26 and O111

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Introduction: Matrix-assisted laser – desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a powerful approach for the rapid identification of microorganisms. Although enterohemorrhagic *Escherichia coli* (EHEC) is an important pathogen that causes severe foodborne diseases it has been challenging to distinguish EHEC and non-EHEC by MALDI-TOF MS. The *S10*-GERMS method has been developed as the strain level discrimination method with high reproducibility and reliability.

Purpose: The purpose of this study was to evaluate the discrimination approach of the major EHEC, serovar O157, O26, O111 and the others by MALDI-TOF MS based on *S10*-GERMS method.

Methods: Totally twenty-nine O157, six O26 and two O111 strains, seven EHEC strains with another serovars, and sixteen non-EHEC strains were obtained from culture collections. First, the observed *m/z* values were obtained by MALDI-TOF MS analysis of the genome-sequenced strains. Second, the theoretical *m/z* values of ribosomal proteins in *S10-spc-alpha* operon were calculated by determination of the DNA sequence or sequence data obtained from NCBI database for genome-sequenced strains and then verified by comparison with the observed *m/z* values. The EHEC-specific protein peaks were selected as biomarkers for the in-house classification tool which has been developed based on *S10*-GERMS method. To verify the accuracy and reproducibility of discrimination with the selected biomarkers, sixteen O157 strains isolated from food samples were analyzed with the various expected sample conditions in daily analysis.

Results: We demonstrated semi-automated pattern matching classification with in-house classification tool with binary matrix using the selected four biomarkers and thirty-seven O157, O26 and O111 strains were successfully classified as proper serovar groups with 100% reliability. Sixteen O157 strains from food samples were successfully determined as O157 by general MALDI-TOF MS analysis either with sinapic acid or CHCA as matrix.

Significance: The discrimination method for major EHEC, O157, O26 and O111 was established based on the *S10*-GERMS method by using four biomarkers observed in MALDI-TOF MS analysis. This approach allows rapid screening of *E. coli* O157, O26 and O111 from the others by a routine MALDI-TOF MS analysis regardless of the sample condition.

P1-14 Towards Development of Effective Interventions to Eliminate *Escherichia coli* during Carcass Chilling

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Introduction: Enteropathogenic *Escherichia coli* are an important cause of foodborne outbreaks involving meat products. Although several treatments, or "interventions", are used to reduce carcass contamination with this pathogen, no single intervention is 100% effective, and additional techniques and technologies are sought. Air chilling of carcasses has been found to reduce pathogen loads.

Purpose: This project aimed to develop novel interventions for *E. coli* on carcasses by studying the effects of temperature and water activity (a_w) changes on carcass surfaces during air chilling to identify whether the lethal effects can be enhanced.

Methods: Exponential phase *E. coli* O157:H7 strain Sakai were subjected to simultaneous rapid downshifts in temperature and a_w, from 35°C a_w 0.993 to 14°C a_w 0.967 in a nutrient broth. Responses of *E. coli* were analysed by viable count; changes in its proteome were characterised by 2D-LC/MS/MS analysis. In subsequent studies, an oxidant (i.e., hydrogen peroxide or chlorine dioxide; both at 75 ppm) was applied at different phases of *E. coli* growth during exposure to the combined shifts (i.e., 1h, 8h and 25h after the shifts) and changes in *E. coli* numbers determined.

Results: Exposure of *E. coli* to abrupt downshifts in temperature and a_w albeit to non-lethal conditions produced a complex pattern of population change which could be divided into three phases including inactivation and recovery phases. Proteomic analysis revealed a transient decrease in several proteins involved in oxidative stress responses during the early response phase. Subsequent trials confirmed that *E. coli* subjected to the environmental shifts became more susceptible to oxidative damage during the early phase of the response when compared to later phases.

Significance: The present results highlight the potential application of oxidants at appropriate time during air chilling to control or eliminate *E. coli* on carcasses.

P1-15 Rapid Typing of BIG 7 Shiga Toxin-producing *Escherichia coli* in Food by Genome Sequence Scanning

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Introduction: The BIG 7 Shiga toxin-producing *Escherichia coli* (STEC) which includes *stx*$^+$ and *eae*$^+$ strains from major serogroups O26, O45, O103, O111, O121, O145, and O157:H7 are classified as beef adulterants by the USDA FSIS. The food industry relies on rapid and sensitive methods to detect these pathogens, thus ensuring a safe supply of foods. Current approved PCR screening methods show high false-positive rates in detecting pathogens. PathoGenetix's automated Genome Sequence Scanning (GSS) system is developed to rapidly and accurately identify pathogens within five hours from enriched samples. GSS system generates restriction DNA fragments from target strains which are fluorescently labeled with sequence-specific probes. The labeled DNA molecules are then linearized within a microfluidic chip, passed through spots of focused laser light, and their fluorescence measured. These sequence-specific optical patterns generated from the genomic DNA fragments are compared to a template database containing more than 500 *E. coli* strains for pathogen identification.

Purpose: To demonstrate typing of BIG 7 STECs directly from produce and meat following enrichment that is compatible with current USDA and industry practices.

Methods: We spiked 25 g samples of spinach or ground beef with TMSTEC plates, respectively.

Results: Of the 96 samples spiked with a strain of STEC, 93 samples (97%) were confirmed to be positive for the correct strain. No false positives were detected.

Significance: The data demonstrates that GSS correctly identifies all BIG 7 STECs in two different matrices using commercial and USDA/FDA recommended workflows.

P1-16 Detecting *Escherichia coli* O157:H7 in Composite 375 g Raw Ground Beef Samples Using the DuPont™ BAX® System Method

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Introduction: Composite sample testing is quickly becoming the industry-accepted method for monitoring the incidence of *Escherichia coli* O157:H7 in non-intact raw beef, including beef trimmings and ground beef. Mandating a representative 60 – 6.25 g sub-samples per lot of beef trim, a 375 g analytical test portion is likely to be implemented for destined raw finished products, which are at further risk for contamination throughout grinding and processing operations.

Purpose: In response to increasing industrial sample sizes, the DuPont™ BAX® System for Real-Time *Escherichia coli* O157:H7 was validated for 375 g analytical portions against the USDA-FSIS MLG 5.07.

Methods: Ground beef patties obtained from local retail outlets (Wilmington, DE) were artificially inoculated with *Escherichia coli* O157:H7. Following a 72 hour cold stress, reference method samples were enriched in modified Tryptic Soy Broth (mTSB), whereas alternative method samples were enriched in mTSB with 2 mg/l of novobiocin (mTSB+n). All samples were incubated at 42 ± 1°C for 12-22 hours, and culture confirmed as outlined in USDA-MLG 5.07 regardless of presumptive PCR result after 22 hours.

Results: PCR testing of mTSB+n enriched samples (n = 30) at 14 and 22 hours accurately detected all 9 culture positives. Similarly, all 15 presumptive positive samples at 14 and 22 hours of enrichment following the USDA reference method were culturally confirmed.

Significance: The data demonstrates that the PCR method for detection of *E. coli* O157:H7 in 375 g ground beef is equivalent to the reference culture method, providing customers the option of analyzing larger composite samples in a rapid manner to further reduce the risk of contaminated finished product.

P1-17 Detection of *Escherichia coli* O157H:7 and Non-O157 Shiga Toxin-producing *E. coli* by Triplex Real-time PCR

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Introduction: Foodborne outbreaks caused by *Escherichia coli* O157H:7 and Non-O157 Shiga toxin-producing *E. coli* (STEC) remain an important food safety concern and a serious public health problem in the United States and worldwide.

Purpose: In order to effectively mitigate future outbreaks caused by this food-borne pathogen, it is necessary to have an ample availability of sensitive, specific, and reliable methodologies to detect *E. coli* O157:H7 and non-O157 STEC strains.

Methods: A triplex real-time PCR was developed for specific detection of *E. coli* O157:H7 by simultaneously targeting a putative fimbriae protein ORF Z3276 and its Shiga toxin (Stx1 and stx2) genes.

Results: The sensitivity of the triplex assay on the target genes of Z3276, *stx1*, and *stx2* was found to be only slightly lower than that of those genes obtained with uniplex PCR assays. The detection limit for this triplex real-time PCR are 100 fg DNA, which is equivalent to less than 20 CFU per reaction. In inclusivity and exclusivity tests containing 182 bacterial strains, all *E. coli* O157:H7 (n = 133) were identified as positive, other strains including non-O157 (n = 40), *Salmonella enterica* (n = 3), and *Shigella* strains (n = 6) were not detected.

Significance: This triplex real-time PCR assay is sensitive and specific, and is useful in detection of *E. coli* O157:H7 and non-O157 STEC.

P1-18 Development of an Internal Standard Approach for Comparing Flow Cytometry-based (FCB) Pathogen Detection System (Rapid-B Pathogen Detection System, Vivione Biosciences) and Quantitative PCR for Enumeration of Foodborne Pathogenic *Escherichia coli*

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Introduction: Shiga toxin-producing strains of *Escherichia coli* (STEC) are of particular concern in food contamination because of the serious clinical pathology caused by this pathogen. Standardization of rapid detection methods for STEC in food samples are needed to ensure a safe food supply.

Purpose: For rapid detection there is a need to have access to internal preset concentrations of the target pathogens that can be used for assessing recovery of cells quantified by the detection method being evaluated. The aims were to compare two bacterial cell fixation methods and to assess them as internal standard cell preparations for quantifying fixed STEC cells by FCB and qPCR. The fixation methods were A) a commercial fixative (Sigma-Aldrich 4% formaldehyde/10% neutral buffer), and B) a fixative used previously (8% formaldehyde, 0.8% NaCl).

Methods: STEC *E. coli* strain O103:H11 was grown for 16 hours in Luria-Bertani medium then fixed for 30 minutes with periodic vortexing. Cells were centrifuged then washed in phosphate-buffered saline twice. Cell concentration was estimated using a Petroff-Hauser counter, and replicate samples were prepared by serial dilution of cells for FCB (Vivione Biosciences) or DNA extraction for qPCR (Eppendorf).

Results: The two methods did not differ significantly in cells quantified by FCB ($P > 0.4$). However, the qPCR method was able to detect as few as 152 cells in 5 microliters of an 8 log dilution using fixative A, but had a detection limit 10 times less sensitive in fixative B.

Significance: The results so far indicate that either fixative method will work for FCB but the fixation method appears to impact qPCR sensitivity. A whole cell-based internal standard solution will allow for comparison of very different rapid methods such as qPCR and FCB for the detection and quantification of *E. coli* STEC cells.

P1-19 The Assessment of *Escherichia coli* as an Indicator of Microbial Quality of Irrigation Waters Used for Produce

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Introduction: *Escherichia coli* is a bacterial species that lives in the gut of all warm-blooded animals, fish, birds as well as reptiles and is commonly used as an indicator of fecal contamination in water.

Purpose: Currently, no microbial indicator standards exist for irrigation waters used for produce production in the US. The produce industry suggests that the recreational water guideline (126 *E. coli*/100 ml) established by the United States Environmental Protection Agency (USEPA) be used. There is concern that the false positive rate of *E. coli* detection may be high in these waters giving false indications of the level of risk from enteric pathogens.

Methods: Three commercially available media for *E. coli* detection were evaluated in Yuma and Maricopa, AZ, and Imperial Valley, CA, and then assessed false positive rates by utilizing polymerase chain reaction (PCR) and DNA sequencing. The media chosen for evaluation were (1) MI Agar, (2) IDEXX Colilert Quanti-Tray® and (3) m-ColiBlue24® broth, accepted by the USEPA and widely used by the produce industry. One-L irrigation water grab samples (n = 450) were collected between March and November 2012. The samples were analyzed for both cultural counts and water quality parameters including temperature, salinity and pH. Isolates positive and negative for *E. coli* were then selected and assessed utilizing PCR and DNA sequencing.

Results: The false positive rate of each method was found to be high, with MI Agar, m-ColiBlue24® broth and the IDEXX Colilert Quanti-Tray® at an accuracy of 67%, 72%, and 51%, respectively. A false positive result is reported when presumptive *E. coli* sub cultured from the media is found to be non-*E. coli* through molecular analysis.

Significance: Overall the IDEXX Colilert Quanti-Tray® performed at a greater rate of accuracy than the other two media evaluated, however, high false positive rates may lead to inaccurate assessment of water quality.

P1-20 Evaluation of ChromID® EHEC Agar for Detection of Seven Major Serogroups of Shiga Toxin-producing *Escherichia coli* from Cattle Feces

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Developing Scientist Competition

Introduction: Seven serogroups (O26, O45, O103, O111, O121, O145, and O157) of Shiga toxin-producing *Escherichia coli* (STEC) account for the majority of foodborne STEC infections. Cattle are asymptomatic reservoirs for STEC. Typically, the organisms colonize the hindgut and are shed in

the feces, which serves as a source of contamination of food products. Culture-based detection and isolation of STEC includes immunomagnetic separation followed by plating on a variety of chromogenic media, some of which are available commercially.

Purpose: We evaluated the performance of two chromogenic media, a chromID® (bioMeriéuex SA, Marcy-l'Etoile, France) and a MacConkey agar-based medium with sucrose, sorbose, novobiocin and tellurite (modified Possé medium; MP) for the detection of seven STEC in cattle feces.

Methods: Fecal samples (n = 6) from beef cattle, spiked with seven serogroups, and pen-floor fecal samples (n = 144) from a feedlot were used in the evaluation. Feces were enriched (6h at 40ºC) in *Escherichia coli* broth and subjected to immunomagnetic separation for each target serogroup. Bead suspensions were spread-plated onto sorbitol MacConkey with cefixime and tellurite (CT-SMAC; for O157), MP (for six non-O157), and chromID® (for seven STEC) media. Chromogenic colonies from MP and chromID® agar and sorbitol-negative colonies from CT-SMAC were tested by multiplex PCR to confirm target serogroups and major virulence genes.

Results: Based on the visual observation of the number of colonies, chromID® appeared to have less crowded and more distinct chromogenic colonies compared to MP medium. A higher percentage of the O45 serogroup was detected from fecal samples with chromID® compared to MP. Additionally, *E. coli* O157 was isolated from 69.4% of fecal samples (144) with chromID® compared to 54.2% with CT-SMAC.

Significance: Data suggested that chromID® were more selective and better for detecting chromogenic STEC, particularly O157 and O45, from cattle feces compared to MP or CT-SMAC medium.

P1-21 Evaluation of Different Immuno-magnetic Beads and Selective Plating Media for the Confirmation of Shiga Toxin-producing *Escherichia coli* Detected by Molecular Methods in Beef Trim Enrichment Broths

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Introduction: Shiga toxin-producing *Escherichia coli* of serogroups O26, O45, O103, O111, O121 and O145 are a testing concern for the beef industry and regulators. Numerous tests are available that attempt to predict the presence of these pathogens. However, potential positive results require culture confirmation entailing immuno-magnetic separation of the targeted pathogen and plating to differential media capable of aiding laboratories in rapidly identifying suspect colonies. Therefore, several commercial products have become available for use in culture confirmation.

Purpose: To compare culture confirmation rates using IMS beads available from Abraxis, BioControl, Dynal, LabM and Romer Labs plated onto six different STEC selective agars (modified Rainbow, R&F STEC media, chromID EHEC media, CHROMagar STEC, washed sheep's blood agar, and STEC Differential Agar).

Methods: Culture confirmation using each IMS bead-media combination was performed on 54 STEC potential positive beef trim enrichments (9 enrichments per O group).

Results: Variations were observed in bead-media combinations in the number of confirmations, levels of background colonies, and number of non-confirming suspect colonies present. Overall BioControl (52%), Abraxis (46%), and Romer (45%) IMS beads confirmed a greater ($P < 0.05$) number of positives than the numbers confirmed by Dynal and LabM beads (41 and 43%, respectively). However, within a serogroup, Dynal-O103, Abraxis-O111, and LabM-O121 beads confirmed more positive cultures than the respective BioControl beads. Modified Rainbow and sheep's blood agars confirmed the greatest numbers of samples overall (57 and 53%, respectively), which were significantly greater ($P < 0.05$) than R&F and STEC differential agars (44 and 38%, respectively). Within serogroup differences were again observed, with equal numbers of O45 confirmed on R&F STEC agar as on modified Rainbow agar.

Significance: The optimal IMS bead and selective media combinations have been identified for use in STEC culture confirmations. (Mentioning product names neither constitutes guarantee/warranty, nor implies approval to exclusion of others.)

P1-22 Validation of a New and Innovative Rapid Assay for the Detection of *Salmonella* in Food and Environmental Samples

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Introduction: *Salmonella* is omnipresent in nature and comprises over 2,500 identified serotypes. It can be found in intestinal tract of vertebrates including livestock, domestic pets and human as well as in the environment. Based on this ubiquity, it is not surprising to find food contaminated with *Salmonella*. Among them, eggs and egg derivatives are commonly identified as being contaminated with *Salmonella.* An effective way to reduce the prevalence is to monitor its presence in the final product and in the environment of the food processing and apply the appropriate corrective measures when necessary.

Purpose: The aim of the study was to develop and validate an immunomagnetic lateral flow assay for the rapid detection (in less than 24h) of the most frequently isolated *Salmonella* (group B to E) in eggs and egg derivative products and in environmental samples.

Methods: The performance was evaluated according to the Guidelines of the AOAC for the Performance Tested Methods℠. Method comparison study was carried out using 600 samples (egg products and environmental surfaces). Half of the samples were analyzed using the new assay and the other half using reference methods.

Results: During the comparison study, the new developed assay correctly identified 299/300 of the samples tested. Difference of probability of detection was used as statistical model to confirm no significant difference between both methods. Ninety-nine percent (102/103) of the *Salmonella* strains tested belonging to serogroups B to E were detected. None of the 31 non-target strains tested shows cross-reactivity in the assay enrichment conditions. In addition, this new detection method shows strong robustness, good stability, consistency and good reproducibility.

Significance: The new FoodChek™ Salmonella assay is an effective tool for the rapid detection of *Salmonella* spp. in whole liquid eggs, liquid egg white, shell eggs, dried whole eggs, dried egg yolks and on environmental surfaces.

P1-23 Evaluation of 3M™ Molecular Detection System (MDS) for the Rapid Detection of *Salmonella* spp. on Duck Wings, Bean Sprouts and Fish Balls

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Introduction: Meat, fresh produce and seafood are documented vehicles of transmission of *Salmonella* to humans. A novel real-time, user-friendly rapid detection system using isothermal DNA amplification coupled with bioluminescence has been recently developed. Studies have been done using this system in the U.S. and Europe; however, more data are necessary for foods produced in Southeast Asia.

Purpose: The objective of this study was to evaluate the performance of the rapid 3M™ Molecular Detection System (MDS) in comparison with the ISO method for detecting healthy, injured and naturally contaminated *Salmonella* spp. on locally produced duck wings, bean sprouts and fish balls.

Poster

Methods: Healthy and injured *Salmonella* spp. were inoculated on food matrix at 10^0 and 10^1 CFU/25g. Injured cells were prepared by heat treatment for duck wings and fish balls, and chlorine treatment for bean sprouts. Un-inoculated samples were used as control along with an additional of 30 naturally contaminated samples as validation for each food matrix. A total of 360 samples were subjected to both the rapid and ISO methods with reported qualitative results compared.

Results: Regardless of inoculum levels, the detection by the rapid method showed 100% sensitivity and specificity for both inoculated and un-inoculated samples compared with the ISO method, except for bean sprout samples, which were inoculated with 10^0 CFU/25g of injured *Salmonella* spp. The validation study also showed that the rapid method could provide 91% sensitivity and 95% specificity for naturally contaminated samples.

Significance: MDS was demonstrated to be a cost effective method, as this system could provide rapid, accurate detection of healthy, injured and naturally contaminated *Salmonella* spp. on duck wings, bean sprouts and fish balls in less than 48 h. This study also suggests that a secondary enrichment may be necessary to enhance the performance of the rapid method for some challenging food matrices with a high microbial load.

P1-24 Performance of Techniques for Identification of Serotypes of *Salmonella*

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Introduction: Traditional identification of the *Salmonella* genus has been routinely done by means of biochemical and serological characteristics. However, in recent years, several countries have focused requirements for poultry products in the control of some specific types of *Salmonella,* such as *S*. Enteritidis and *S*. Typhimurium. In this case, the product in which the presence of *Salmonella* is detected, the bacteria must be identified in some way to prove the presence or not of these serotypes.

Purpose: The purpose of this study was to compare different techniques for the identification and differentiation of *Salmonella* spp.

Methods: Thirty-five isolates of *Salmonella* obtained from chicken products were submitted to identification by classical serological, automated biochemistry VITEK 2 system (bioMérieux), mass spectrometry - MALDI-TOF Bio Typer (Bruker Daltonik), and by genetic analysis by ribotyping with microbial characterization RiboPrint® system (DuPont Qualicon).

Results: The serological technique identified the following serotypes: two *S*. Saintpaul, one *S*. Muenchen, two *S*. Mbandaka, two *S*. Newport, two *S*. Infantis, two S. Senftenberg and 24 *S*. Enteritidis. All 24 isolates identified as *S*. Enteritidis by serological technique were also confirmed by the system. Two isolates of *S*. Saintpaul and one of *S*. Senftenberg obtained in the serological technique were identified in the system as *S*. Typhimurium. The other isolates were identified only on species level. Using mass spectrometry all isolates were identified only as *Salmonella* spp. The 15 isolates tested by RiboPrint® were *S*. Enteritidis and the system confirmed the results.

Significance: The results obtained suggest that the system is reliable to identified *S*. Enteritidis, but limited for the others serotypes tested. The RiboPrint® showed high correlation with serological technique, although only isolates of *S*. Enteritidis were tested. Mass spectrometry can be a promising technique for the identification of types of *Salmonella* but it requires further studies.

P1-25 Accuracy of Molecular Screening Methods for the Detection of *Salmonella enterica* in Production Ground Poultry Samples

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Introduction: Accurate detection methods are essential in the poultry industry to detect *Salmonella enterica* in ground poultry products as a means to monitor baselines and verify process controls. Accuracy may be impacted by factors including sample preparation and detection method utilized.

Purpose: The purpose of these studies was to comparatively evaluate the accuracy of the Atlas Salmonella SEN Detection Assay and BAX Salmonella Assays (original and real-time) for the detection of *Salmonella enterica* in production ground poultry products.

Methods: Ground poultry products, consisting of ground turkey (n = 39) and ground chicken (n = 4), were collected by two poultry processors. Sample weights ranging from 25 to 325g were enriched utilizing Buffered Peptone Water (BPW) in a 1:10 dilution at 35° ± 2°C. For the Atlas method, 400 µl was transferred into sterile sample transfer tube at 12 hours (Processor B) and at 18-24 hours (Processor A). Both processors conducted BAX analysis as per routine procedure on the paired enrichments at 20-24 hours. Duplicate 1.5-2.0 ml aliquots from the enrichments were sampled into sterile vials, maintained at 4°C, and shipped to Roka for cultural analysis. Atlas and culture results were reported to collaborators at which time BAX results were disclosed.

Results: In total, *Salmonella enterica* was identified in 27.91% of samples by culture analysis, in 25.58% by the Atlas assay and in 16.28% by the BAX assay. The BAX assay reported 5 false negative results and the Atlas assay report 1 false negative result as compared to culture on ground poultry samples. Percent agreement of the screening tests to culture for the Atlas and BAX Salmonella assays were 97.67% and 88.37%, respectively.

Significance: Screening method performance and accuracy can be adversely affected by matrix characteristics, variation in sample preparation procedures, and the rapid detection method utilized.

P1-26 Accuracy of Molecular Screening Methods for the Detection of *Salmonella enterica* in Production Poultry Rinse Samples

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Introduction: Accurate detection methods are essential in the poultry industry to detect *Salmonella enterica* in whole carcass rinse samples as a means to monitor prevalence and verify process controls. Variability in sample preparation and detection method may influence accuracy of results.

Purpose: The purpose of these studies was to comparatively evaluate the accuracy of the Atlas Salmonella SEN Detection Assay and the BAX Salmonella Assay for the detection of *Salmonella enterica* in carcass rinse samples from multiple processors.

Methods: Three poultry processors collected routine rinse samples according to USDA FSIS MLG 4.07 by rinsing each carcass with ~400 ml of Buffered Peptone Water (BPW). A 30 ± 0.6 ml post rinse aliquot was combined with 30 ± 0.6 ml of sterile BPW and enriched for 20-24 hours at 35° ± 2°C. Each sample was analyzed by Atlas, BAX, and culture methods. For the Atlas method, 400 µl of enrichment was transferred into transfer tubes. For the BAX method, 1.5-2 ml of enrichment was transferred into sterile cryovials and held at 4°C. Samples were processed at Roka on the Atlas according to the approved Atlas method and by culture according to MLG 4.07. Processors sampled paired enrichments for BAX analysis according to routine procedure. BAX results were disclosed after Atlas method and culture results were reported.

Results: The Atlas method reported 1 false negative, whereas the BAX method reported 9 false negative and 1 false positive results compared to culture. Percent agreement between culture and Atlas and BAX Salmonella assays were 99.31% and 93.06%, respectively. Processors A, B and C contributed 25.69, 27.78 and 46.53% of 144 samples. All discrepant results were attributed to the 25.69% of samples prepared by processor A.

Significance: Screening method performance and accuracy may be adversely impacted by the proficiency or complexity of operator handling requirements.

P1-27 Method Verification for the Detection of *Salmonella enterica* by the Atlas® *Salmonella* G2 (SG2) Detection Assay in Produce Matrices

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Introduction: *Salmonella enterica* has been implicated in over 1 million cases of salmonellosis annually in the United States according to the Centers for Disease Control and Prevention (CDC). Consequently, FDA has increased its attention on produce safety, and FSMA regulations may increase testing volumes thereby necessitating accurate and rapid methods to provide confident and timely results to the produce industry.

Purpose: The purpose of this study was to verify performance of the Atlas® *Salmonella* G2 (SG2) Detection Assay on additional produce matrices not previously submitted for AOAC-RI validation.

Methods: Produce matrices evaluated were spinach, iceberg lettuce, red leaf lettuce, fresh blueberries and scallions. For each matrix, six 25 g samples and one 375 g sample were prepared and inoculated with ~ 8 CFU/sample of *Salmonella* Newport (Cornell S5-436), and six 25 g samples were prepared as uninoculated matrix controls. All samples were enriched with Universal Pre-enrichment Broth (UPB) at a 1:9 sample to media ratio and incubated at 42 ± 2°C for 10 and 24 hours. Samples were collected at 10 and 24 hours according to the assay product insert and loaded onto the Atlas instrument. All samples collected at 24 hours underwent culture confirmation according to the FDA BAM Chapter 5 reference method.

Results: The Atlas method specifically detected *Salmonella enterica* in all inoculated samples at 10 and 24 hours and were culture confirmed. All uninoculated samples were negative according to the Atlas method at 10 and 24 hours and culture at 24 hours.

Significance: The Atlas method was verified for the propagation and detection of *Salmonella enterica* in five additional produce matrices using a 10 hour enrichment and total time to result of 13.5 hours with no false positive, false negative or inhibited results. The results substantiate the efficiency and accuracy of the assay on foods outside the current AOAC-RI approved matrices.

P1-28 Evaluation of the 3M™ Petrifilm *Salmonella* Express System for the Detection of *Salmonella* in Thailand Beverages

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Introduction: With ongoing consumer consciousness of "healthful" and "functional" foods, the beverage industry in Thailand has seen continuous volume growth. Domestic regulations are in place to prevent and control *Salmonella* contamination in beverage products, and the industry has a demonstrated need for faster and simpler detection methods to comply with these regulations.

Purpose: This study evaluated the performance of 3M™ Petrifilm™ *Salmonella* Express (SALX) System (an all-in-one method providing qualitative detection and biochemical confirmation) for the detection of *Salmonella* in a variety of beverages from Thailand as compared to the standard cultural method ISO 6579:2002 /Amd. 1:2007.

Methods: Products (n = 134) were purchased as manufacture-sealed packaging at retail counters, markets, and street vendors. Matrices included carbonates, fruit/vegetable juices, functional beverages, concentrates, powdered and ready-to-drink tea & coffee. Thirty-three of these samples were artificially inoculated with one of three different *Salmonella* spp. (*S*. Typhimurium, *S*. Rissen and *S*. Stanley) and the remaining samples were tested uninoculated. Approximately 100 ml or 100 g of each sample was first homogenized and then analyzed following two methods; 3M™ Petrifilm™ SALX and ISO 6579:2002 /Amd. 1:2007.

Results: The 3M™ Petrifilm™ SALX showed comparable detection results to those obtained from the ISO method. The sensitivity of the 3M™ Petrifilm™ SALX was 100% (95% CI : 95.4-100 %), specificity was 100.0% (95% CI : 95.4-100 %), accuracy was 100.0% and no significant difference from the reference method using the chi-square statistic.

Significance: Rapid detection and monitoring of *Salmonella* in beverage products can help to improve beverage safety and quality in domestic and international markets. The 3M™ Petrifilm™ *Salmonella* Express System is a new all-in-one detection method that can facilitate high-throughput sample screening for *Salmonella* to obtain fast and reliable results.

P1-29 Evaluation of the 3M™ Molecular Detection Assay (MDA) *Salmonella* for the Detection of *Salmonella* in 125 g of Thai Vegetables

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Introduction: Thai vegetables are widely acknowledged by consumers as important for a healthy diet. However, they have a relatively short shelf life and are often consumed raw without heat treatment. Therefore, the Thailand Plant Standard and Certification Office (whose function includes pre-export certification) requires fast and sensitive test methods for the early detection of *Salmonella* in fresh vegetables.

Purpose: To evaluate the performance of a new *Salmonella* molecular detection method through comparison with a cultural method and an ELFA method for Thai vegetable matrices.

Methods: A total of 115 samples were tested: 35 samples were artificially contaminated with a low level of *Salmonella* (1.9-6.6 CFU/test portion); 30 samples were artificially contaminated with a medium level of *Salmonella* (31-36 CFU/test portion); 50 samples were tested without artificial contamination. After a shared enrichment (125g test portion diluted 1:10 in BPW at 37+1°C for 18-24h), all samples were analyzed by three methods: (a) 3M™ Molecular Detection Assay (MDA) *Salmonella* (modified); (b) ELFA method and (C) ISO 6579. The modified 3M method involved a secondary RVS Broth enrichment incubated at 41.5+1°C for 22-24 hours. Presumptive positive molecular results were culturally and biochemically confirmed following ISO 6579 procedures.

Results: For the 115 samples tested, the 3M MDA *Salmonella* method showed 100% sensitivity, 97% accuracy, 90% specificity while the ELFA method showed 96% sensitivity, 97% accuracy, 100% specificity. No statistical difference between the 3M MDA *Salmonella* method and reference method can be detected using the McNemar test and the 3M MDA *Salmonella* method reported a relative detection level of 0-1.9 CFU/test portion.

Significance: The 3M™ MDA *Salmonella* offers sensitive and reliable results for the detection of *Salmonella* in 125g of artificially and naturally contaminated Thai vegetables and offers significant advantages for exporters of Thai vegetables, including faster time to results.

P1-30 Comparison between 3M™ Petrifilm™ *Salmonella* Express System and ISO 6579 Conventional Method for the Detection of *Salmonella* spp. in Cook-chill and Cook-freeze Retail Foods in Bangkok, Thailand

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Introduction: Cook-chill and cook-freeze foods have become increasingly common in Bangkok with consumers purchasing these foods via caterers, supermarkets and restaurants. Despite this increasing popularity, the microbial safety of these products — specifically *Salmonella* contamination — has not been studied extensively

Purpose: The aim of this study is to determine natural contamination rates of *Salmonella* in cook-chill and cook-freeze foods sold in Bangkok. Additionally, this study aims to evaluate the performance of 3M™ Petrifilm™ *Salmonella* Express (SALX) System for the detection of *Salmonella* spp. in various types of cook-chill and cook-freeze foods compared to the standard ISO 6579 conventional method

Methods: Cook-chill (26 samples) and cook-freeze (74 samples) foods were randomly purchased from supermarkets in Bangkok. Fifty grams of each sample was homogenized, divided equally and analyzed following two methods: (1) 3M™ Petrifilm™ SALX System; (2) ISO 6579 using BPW pre-enrichment, MKTTn & RV selective enrichment, and XLD & HE agar for selective plating. Furthermore, five replicates of six different matrices (*Salmonella* negative samples, n = 30) were spiked with low levels (1–10 CFU/25g) of *Salmonella* Typhimurium to validate the performance of 3M™ Petrifilm™ SALX System compared to the standard ISO 6579 conventional method.

Results: Nine samples (8.22%) of cook-freeze foods were naturally contaminated with *Salmonella* spp. (five samples were positive by both methods; three samples were positive by ISO method only; one sample was positive by 3M™ Petrifilm™ SALX System only). All cook-chill samples were negative (0% natural contamination). For the artificially inoculated samples the relative accuracy, relative sensitivity and relative specificity of the 3M™ Petrifilm™ SALX System was found to be 96.9%, 92.1% and 98.9%, respectively.

Significance: The data from this study supports the use of 3M™ Petrifilm™ SALX System as a rapid and cost-effective method for *Salmonella* detection in cook-chill and cook-freeze foods.

P1-31 Performance of a New Molecular Platform for the Detection of *Listeria monocytogenes* in Thai Seafood Matrices

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Introduction: *Listeria monocytogenes* is a widely recognized hazard in the food industry, associated with many types of food, (including seafood). Thailand's exports of fresh, chilled and frozen seafood products stand at around 570,000 metric tons per annum with production centralized around the Samutsakorn and Samutsongkhram provinces. Pre-export certification and inspection is provided by the Samutsakorn Fish Inspection and Research Center (SFIRC) [part of the Department of Fisheries], however, current test methods for pathogens are perceived as complicated and lengthy. For the expansion of the Thai seafood export industry, SFIRC has prioritized the qualification of molecular methods for rapid *Listeria monocytogenes* detection.

Purpose: To evaluate the performance of a molecular method for the detection of *Listeria monocytogenes* through comparison with a cultural method for Thai seafood matrices, including [1] Raw Fish, [2] Raw Shrimp, & [3] Raw Octopus.

Methods: A total of 120 samples were analyzed; 60 samples were artificially contaminated with a cocktail of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*; 60 samples were tested with natural contamination. After enrichment in Demi Fraser Broth Base and Fraser Broth Base, the samples were analyzed by 3M™ Molecular Detection Assay *Listeria monocytogenes*, and a cultural method following the US FDA Bacteriological Analytical Manual (BAM). Presumptive positive molecular results were culturally and biochemically confirmed following BAM protocols.

Results: For the 120 samples tested, the molecular method showed 100% sensitivity, 100% accuracy, 100% specificity and no significant difference from the cultural method using the chi-square statistic.

Significance: Testing artificially and naturally contaminated seafood matrices from Thailand, the 3M Molecular Detection Assay *Listeria monocytogenes* was found to be sensitive and accurate. Other benefits were noted — including faster time-to-results — that demonstrate the significance of this evaluation for the Thai seafood export industry.

P1-32 Evaluation of a New Low-cost Multicolor Fluorescence Capillary Electrophoresis System for Multiple-locus Variable-number Tandem-repeat Analysis (MLVA) of *Salmonella*

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Introduction: MLVA is a powerful emerging method for molecular subtyping of pathogens and a possible replacement for the "gold standard," PFGE. MLVA is easy to perform and interpret, highly discriminatory and reproducible. However, a major drawback is that MLVA is currently performed using prohibitively expensive four-color Sanger sequencers, limiting wider use of this approach.

Purpose: Availability of a low-cost, yet capable MLVA platform could unlock the potential for wider use of this typing approach. We sought to evaluate the performance of a new multicolor capillary electrophoresis (CE) instrument (the MLVAnalyzer, Advanced Analytical Technologies, Ames, IA) for MLVA-based analysis of *Salmonella*. Specifically, we sought to evaluate the system's discriminative ability and reproducibility using a panel of *Salmonella* strains.

Methods: Fluorescent dye-labeled primers (TAMRA, HEX, TET) targeting five variable number tandem repeat (VNTR) loci on the *Salmonella* genome were prepared and genomic DNA was extracted from various strains of *Salmonella* Typhimurium, Heidelberg and Enteritidis. Multiplex PCRs were performed, generating five differently-sized PCR products for each strain. Amplified fragments were separated by CE in the MLVAnalyzer and were analyzed by size and color using the system's software.

Results: Multiplex PCR targeting short tandem repeats (STRs) yielded products ranging in size from ~150 to 500 bp. These were detected by the MLVAnalyzer as they passed in front of the system's continuous linear filter using distance-phased color multiplexing and were differentiated using onboard software. The MLVAnalyzer provided accurate and reproducible differentiation of *Salmonella* strains, and its digital output facilitated database development and dendrogram-based analyses of *Salmonella*.

Significance: Our results indicate that the MLVAnalyzer can provide reliable strain typing data suitable for outbreak investigations and microbial source tracking. Availability of a lower-cost MLVA platform may help unlock the potential for wider use of MLVA by smaller laboratories as a capable alternative to PFGE.

P1-33 The ANSR® *Salmonella* Assay for the Detection of *Salmonella* spp. in Pasteurized Egg Products

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Introduction: ANSR® *Salmonella* is a new molecular diagnostic assay based on the nicking enzyme amplification reaction for detection of *Salmonella* spp. in foods and environmental samples. Following single-step enrichment, the assay is completed within 30 minutes, including sample preparation.

Purpose: The purpose of the study was to evaluate performance of the ANSR *Salmonella* method for detection of *Salmonella* spp. in pasteurized egg products, including dried whole egg, dried egg white, frozen liquid egg yolk and liquid whole egg, in comparison to that of the reference culture method of USDA-FSIS.

Methods: For both the ANSR and reference methods, 20 test portions of each egg product were inoculated with *Salmonella* spp. at the level to produce fractional positive results, along with 5 high level samples and 5 uninoculated controls. The samples were held under conditions intended to simulate natural contamination with sub-lethally injured cells. Half of the test portions were analyzed by the ANSR method (for dried egg products, 1:10 dilution in PBS is necessary) after 24h enrichment and half by the reference procedure.

Results: All high level samples tested positive and all negative controls tested negative for each pasteurized egg product by both ANSR assay and reference method. At the fractional positive level, a total of 80 test portions were analyzed by each method. There were a total of 44 positive results by the ANSR assay and 47 by the reference procedures. There were no statistically significant differences in the number of positive results obtained with the ANSR and reference methods for any of the products tested as determined by probability of detection analysis.

Significance: ANSR® *Salmonella* is a rapid, reliable method for detection of *Salmonella* spp. in pasteurized egg products.

P1-34 Heat Resistance Stability of *Enterococcus faecium* NRRL B-2354 and *Salmonella* Enteritidis PT30 on Almonds during Extended Storage at 4 and 25°C

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Introduction: Almond Board of California published protocols for the preparation, storage, and heat resistance verification of *Enterococcus faecium* NRRL B-2354 on inoculated almonds prior to use as a surrogate for *Salmonella* Enteritidis PT30. The protocol specifies storage at a temperature of 4°C for up to 14 days followed by heat treatment at 280°F for 15 minutes in a Fisher Scientific Isotemp 851F oven (Forced Air) to verify heat resistance. This study investigates extension of the specified shelf life.

Purpose: The purpose of this study was to evaluate the change in heat resistance of *Enterococcus* and *Salmonella* on inoculated almonds during 60 days of storage at 4 and 25°C.

Methods: Almonds were inoculated with *Enterococcus* or *Salmonella*, and stored at 4 or 25°C. Over 60 days of storage, inoculated almonds were sampled periodically and heat-treated using Forced Air (280°F/15 minutes) and Gravity Ovens (300°F/15 minutes). Log reductions following heat treatment for each storage condition and sampling time (n = 6) were compared using ANOVA.

Results: Log reductions of *Enterococcus* following both oven treatments over 60 days of storage were not significantly different ($P > 0.05$) when inoculated almonds were stored at 4°C, and ranged between 0.91 ± 0.55 and 1.84 ± 0.82 logs (n = 6), or 0.25 ± 0.12 and 0.83 ± 0.43 logs (n = 6) for Forced Air and Gravity Oven treatments, respectively. Conversely, differences between log reductions of *Enterococcus* on almonds were statistically significant ($P < 0.05$) for both oven treatments over 60 days of storage at 25°C. No significant differences were observed for *Salmonella* on almonds stored at either 4 or 25°C over a period of 60 days in either oven type.

Significance: Results indicate that the current shelf life of almonds inoculated with *Enterococcus* can be extended to 60 days at 4°C without significant impact to the heat resistance of this organism.

P1-35 Pulsed-field Gel Electrophoresis Subtyping for *Salmonella* Serotype Discrimination

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Introduction: Traditional serotyping has been widely used for *Salmonella* identification, indeed more than 2,500 *Salmonella* serovars have been identified through this method. Nevertheless, its limited discriminatory ability has led to the development of different genotypic-based techniques. Pulsed-field gel electrophoresis (PFGE) is considered the "gold standard" *Salmonella* subtyping method; and due to its great discriminatory ability it plays a key role in the determination of clonal relatedness between isolates and in identifying genetic diversity and distribution of pathogens in different environments.

Purpose: This study aimed to analyze the relationship among *Salmonella* serotypes obtained by the traditional serotyping method and PFGE patterns from beef carcasses and feedlot fecal isolates, to assess genetic diversity and determine the spread of different clones in and between carcasses in an attempt to improve interventions at different points in the food chain.

Methods: A total of 95 *Salmonella* isolates, 81 from beef carcasses (hide, pre-evisceration, pre-cooler and cooler) and 14 from feedlot were used in this study. PFGE was performed according to the "One-Day Standardized Protocol for Molecular Subtyping of *Salmonella* serotypes" of PulseNet. Cluster analysis was performed by using Bionumerics software v. 6.6. The isolates that were found clustered with a different serotype than the one obtained in the first traditional serotyping were sent for a second serotyping to the National Veterinary Services Laboratories in Ames, Iowa.

Results: The set of 95 *Salmonella* isolates were typable by PFGE; 26 unique PFGE patterns were identified with 42.3% ($n = 11$) of them corresponding to a single serovar. High concordance (81.8%) was found between the PFGE predicted serotype and the serotype based on the Kauffman-White serotyping scheme.

Significance: The findings of this study suggest PFGE is a suitable method for serotyping discrimination and reflect the need of improving interventions at different points in the food chain to avoid further *Salmonella* spread among carcasses.

P1-36 A Comprehensive Comparison of the BAX® System *Salmonella* 2 and Real-time *Salmonella* PCR Assays to the FDA BAM Reference Methods for the Detection of *Salmonella* in a Variety of Soy Ingredients

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Introduction: There have been several well publicized outbreaks of salmonellosis that resulted from the consumption of contaminated low water activity, ready-to-eat foods with the root cause of some of these outbreaks being traced to the low water activity ingredients. For example, the *Salmonella* outbreak associated with contaminated peanut butter ingredient resulted in a class I product recalls from over 360 firms. Following this

outbreak, the industry and regulators realized the need to evaluate rapid automated pathogen testing technologies in low moisture products and ingredients.

Purpose: The purpose of the evaluation was to proactively evaluate the performance of the BAX System® *Salmonella* 2 and Real-Time *Salmonella* PCR assays using Buffered Peptone Water (BPW) as a primary enrichment against the FDA BAM or ISO 6579 reference methods on a variety of low moisture, soy ingredients: Isolated Soy Protein (ISP), Soy Fiber (SF), Soy Fluid Lecithin (SFL), Soy Deoiled Lecithin (DL), and Soy Nuggets (SN).

Methods: For each method, 20 replicates, either 25g or 375g, were analyzed at one inoculum level: 0.2-2 CFU/test portion. Five control replicates were analyzed at 0 CFU/test portion. Each matrix was analyzed by the candidate method after 22-24 hours of enrichment in BPW with a 3 hour re-growth step in BHI.

Results: The results of a McNemar's Chi-square analysis indicated no statistically significant difference observed between the candidate method and the reference methods ($X^2 < 3.84$). There was no observed reduction of sensitivity in larger 375 g composite samples.

Significance: These studies indicate that the new methods, in combination with the single BPW primary enrichment and subsequent BHI re-growth step, demonstrated equivalent sensitivity and robustness when compared to the reference methods for both 25 g and 375 g composite samples. The new methods also provide a 30h time to presumptive results as compared to up to 3-4 days for the reference methods.

P1-37 Analysis of *Salmonella* spp. Enrichment for Foodborne Pathogen Detection

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Introduction: For successful prevention of foodborne illness, rapid and reliable methods are needed for pathogen detection. Depending on the food matrix, varying pre-enrichment broths are used by FDA field labs as outlined in the Bacteriological Analytical Manual (BAM) for individual pathogen detection. The use of multiple pre-enrichment broths is labor intensive and a roadblock for efforts in multi-pathogen detection screening methods in foods or environmental swabs.

Purpose: This study aims to characterize growth of *Salmonella* spp. in candidate enrichment broths in an effort to identify a universal enrichment broth capable of supporting growth of multiple *Salmonella* serovars.

Methods: Candidate broths included current BAM broths, a FDA research broth (BMW) and modifications of these broths. *Salmonella* spp. (Agona, Bareilly, Braenderup, Brandenberg, Enteritidis, Javiana, Montevideo, Newport, Poona, Saintpaul, Tennessee and Typhimurium) were individually inoculated into candidate enrichment broths to achieve a final concentration of approximately 5 CFU/ml. Growth was analyzed in an automatic growth curve analyzer at 37°C for 24h with OD_{600nm} readings taken every 20 minutes. After 24h of incubation, samples were plated on TSA and XLD. Lag times and maximum growth rates were calculated from OD_{600nm} using DMFit.

Results: After 24h, all *Salmonella* spp. growth was > 9 log CFU/ml in all broths but growth rates varied by serovar and broth. BMW and UBP broths had the lowest lag times, 4.16 ± 0.93h for *Salmonella* Saint Paul in BMW and 3.69 ± 0.14h for *Salmonella* Braenderup in UPB, indicating the fastest growth. *Salmonella* Typhimurium had the highest lag times for all broths, ranging from 7.65 ± 0.43h to 11.83 ± 0.05h.

Significance: Identification of the vital constituents for *Salmonella* growth in enrichment broths will ultimately be used for formulation of a universal enrichment broth enhancing detection of multiple *Salmonella* spp. in food and environmental samples.

Poster

P1-38 A Novel Real-time PCR Method for Rapid Molecular Detection and Serotyping of *Salmonella* Typhimurium

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Introduction: In Europe, food safety regulation was recently reinforced to prevent poultry meats adulterated with either *Salmonella* Enteritidis or *S.* Typhimurium from being marketed. While the current Kauffmann-White (K-W) serotyping method requires 4/5 days to assess the presence of these pathogens, two alternative real-time PCR methods have been developed, allowing their specific detection within one day. As the *S.* Enteritidis method, the new *S.* Typhimurium method described here includes broth enrichment and DNA extraction method and can be used either as a rapid serotyping step after the detection of *Salmonella* spp. or as a direct combined screening & serotyping method. In order to optimize the specificity of the assay, a dedicated method of analysis was developed, based on a dual target PCR system.

Purpose: This study aimed at evaluating key performances of this new real-time PCR *S.* Typhimurium kit on food and environmental samples: the intrinsic detection limit (IDL), and the inclusivity & exclusivity.

Methods: For estimation of IDL, serial dilutions of pure cultures of ATCC14028 *Salmonella* Typhimurium, were subjected to both *Salmonella* spp. and *S.* Typhimurium methods. IDL was defined as the concentration at which 9 out of 10 independent extracts were positive. For inclusivity and exclusivity, this study includes both pure strain extracts and spiked environmental and food samples extracts.

Results: IDL was estimated at 250 CFU/ml. All the 105 inclusivity *Salmonella* Typhimurium strains were tested positive. 96% of the exclusivity samples (including 339 *Salmonella* and 46 non-*Salmonella* strains) yielded negative results.

Significance: Together with the *S.* Enteritidis method, this novel method will allow a faster release of the products and a significant reduction of the number of samples to be confirmed by the standard K-W method.

P1-39 Multiplex PCR for Simultaneous Identification of 8 Common *Salmonella* Serovars

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Developing Scientist Competition

Introduction: *Salmonella* is a significant public health and food safety issue. This organism is the leading cause of foodborne illnesses by bacterial pathogens in the United States. Identifying this pathogen rapidly and accurately helps locate the source of contamination, implement corrective actions in a timely manner, and therefore ensure food safety for consumers and reduce economic loss for industry. PCR has been commonly used as a detection tool for *Salmonella;* however, in most cases, it should be combined with other techniques for further serotyping of isolated *Salmonella*.

Purpose: In this study, we developed a multiplex PCR assay to simultaneously identify eight different *Salmonella enterica* serovars, which have been most commonly associated with foodborne illnesses, including Enteritidis, Typhi, Javiania, Typhimurium, Newport, Gaminara, Michigan, and Agona.

Methods: Fifteen genetic loci were selected, and 17 primers were designed using Bioinformatics software and tested for multiplex PCR development. The final assay was run with 9 primers as two five-plex PCRs, and tested with various *Salmonella* serovars and common foodborne pathogens.

Results: The developed multiplex PCR could correctly identify the tested 8 serovars based on their unique amplification patterns. The results were reproducible and the developed assay did not show any cross-reactivity with other common foodborne pathogens or environmental bacteria. When the assay was tested with experimentally contaminated meat samples, the assay could identify the *Salmonella* serovars without any significant loss of sensitivity.

Significance: This study demonstrated the developed multiplex PCR assay could be a simple and rapid method for molecular subtyping of *Salmonella enterica* in food samples.

P1-40 Detection of *Salmonella* species by the DuPont™ BAX® System Real-time PCR Assay for *Salmonella*: Collaborative Study

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Introduction: *Salmonella* is found in many food and environmental sources and can cause serious illness. Since isolation by culture is long and difficult, especially in the presence of competing flora, rapid methods for its detection are needed.

Purpose: Internal validation studies were previously performed by DuPont Nutrition & Health on 24 matrices to demonstrate the reliability of the test method among a wide variety of sample types. Two of these matrices –frankfurters and orange juice– were each evaluated in 14 independent laboratories as part of a collaborative study to demonstrate repeatability of the method independent of the end user.

Methods: Samples were prepared at three inoculation levels - unspiked, low (<2.0 MPN/test portion) and high (~5 MPN/test portion) – then blind-coded and sent to each participating site. Frankfurters were compared to the USDA FSIS reference method in a paired study design, and orange juice was compared to the FDA BAM reference method in an unpaired study with enrichment in proprietary BAX® System MP media.

Results: For low-spike samples, the BAX® System method returned presumptive positive results for 186 of the 336 samples tested, and the reference method confirmed 182 positive results. For high-spike samples, the BAX® System method returned positive results for all 336 samples tested, and the reference method confirmed all 336 positive results. Also, all negative control samples (n = 336 samples total) were presumptively and culturally negative. At each inoculation level and for each matrix, results from the BAX® System method and the reference method were statistically indistinguishable when analyzed using the Probability of Detection (POD) statistical treatment as required by AOAC.

Significance: This study indicates that PCR detection of *Salmonella* using the BAX® System method is rapid and sensitive. Test kit results demonstrated no significant difference when compared with the reference culture methods.

P1-41 Rapid Serotyping Method to Characterize *Salmonella enterica* subsp. *enterica* Isolates

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Introduction: *Salmonella* is one of the most common enteric pathogens linked to foodborne illness. ProvLab Alberta uses serological methods and follows the White-Kauffmann-Le Minor scheme for serotyping *Salmonella* isolated from food samples during foodborne illness investigations. Phenotypic serovar determination is often incomplete and confirmation may be required at the National Microbiology Laboratory (NML). The Check&Trace Salmonella (CTS) platform (Check-Points, The Netherlands), a commercial DNA microarray system which can fully genetically determine the serotype designation of a known *Salmonella* isolate.

Purpose: We evaluated the CTS platform for genetic characterization of *Salmonella* isolates in parallel to conventional serotyping.

Methods: *Salmonella* subsp. *enterica* identified by conventional methods using commercial (Statens Serum Institute) or in-house antisera were tested on CTS. Isolates were assigned a 'genovar' number based on the microarray result and matched to known serovars in a database.

Results: A total of 471 *Salmonella enterica* subsp. *enterica* isolates were serotyped by conventional methods; 406 isolates had a complete antigenic formula and serovar determined. Of these, CTS found a concordant serovar result to 349 (86.0%), discordant result to 4 (<1%), and a genovar only to 53 (13.1%) isolates. All but seven of the isolates in the "genovar only" category represent serovars rarely found in the top 20 *S. enterica* subsp. *enterica* in ProvLab. Of the remaining 65/471 isolates for which the serovar could not be determined through conventional methods (due to incomplete antigenic formula), CTS was able to determine the majority (54/65, 83.1%) of serovars. These serovars were: 4,[5],12:i:- (29); Enteritidis (2); Heidelberg (2); Javiana (5); Paratyphi B var Java (3); Stanley (5); Typhimurium (3); and others (5).

Significance: CTS is an effective, rapid and easy to use platform and can generate *Salmonella* serovar results within eight hours. Using this molecular typing method can shorten the turn-around-time required during foodborne illness investigations.

P1-42 Withdrawn

P1-43 Pathatrix Auto™ - the First AFNOR-approved Real-time PCR Method for Detecting *Salmonella* in Pooled Food Samples

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Introduction: The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process as many as ten individual food enrichments in the same sample pool. This sampling format has never been approved in the EU market, and would require extensive validation efforts by an expert testing lab to evidence that the approach is not only possible, but practical.

Purpose: In order to validate this product for food safety testing in the EU, this workflow would need to demonstrate a relevant relative detection limit, show statistical similarity to the ISO 16140 reference through accuracy, sensitivity, and specificity; and prove its robustness and practicability in the field.

Methods: Adria Developpement was selected to perform the evaluation to ascertain the Pathatrix Auto's ability to detect *Salmonella*in pooled food sample types by Real-time PCR and selective agar plating. A Ring Trial proficiency study with 15 independent food safety testing labs was also conducted to verify that the workflow was functional and accurate with minimal training.

Results: In both the Adria Developpement study and the Ring Trial, the candidate and reference methods were found to be statistically similar. Of the 202 different food sample types tested during this evaluation, a relative accuracy of 93.1%, a relative sensitivity of 87.7%, and a relative specificity of 96.7% was attained. The relative detection limit was determined to be 0.4-1.5 log CFU/25g of sample, which was statistically similar to the reference. The selected Ring Trial labs demonstrated 100% proficiency and accuracy in performing the workflow.

Significance: This is the first validated method for sample pooling in the EU. The demonstrated robustness, accuracy, and ease of use of this workflow enables the user to rapidly screen for rare contamination events with high confidence, with up to a 90% cost savings over other PCR-based platforms.

P1-44 Detection of Newly Described *Listeria* Species by Three Chromogenic Agars and One Real-time PCR Method

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Introduction: *Listeria* species are indicator organisms for the hygienic management of food processing environments. The presence of the non-pathogenic species of the genus is indicative of poor sanitation practices and possible presence of pathogens. Recently, four new non-pathogenic *Listeria* species were described: *Listeria rocourtiae*, *Listeria marthii*, *Listeria weihenstephanensis* and *Listeria fleischmannii.*

Purpose: The RAPID and iQ-Check ranges of products provide effective methods for the detection of *Listeria* species. They are based on an enrichment step in Half-Fraser broth or *Listeria* Special Broth and subsequent detection on a chromogenic or with real-time PCR. A study was conducted to verify the ability of these methods to properly detect the new *Listeria* species.

Methods: The growth of heat stress cells in LSB and Half-Fraser broth was checked in a bioscreen or by spotting the cultures on TSA. Recovery rates of the chromogenic media were calculated using TSA as the non-selective medium and were compared to those obtained with PALCAM reference agar. The color of the colonies produced on each medium was recorded. An inclusivity/exclusivity study was performed with the PCR kits.

Results: It was demonstrated that LSB was superior for recovery of stress cells. *Listeria rocourtiae* DSM22097, *Listeria marthii* DSM23813 and *Listeria fleischmannii* DSM24998 was detected on all chromogenic media after 24 hours of incubation with a recovery rate > 0.5. *Listeria weihenstephanensis* DSM24698 needed 48 hours to grow. The four strains developed typical colonies of *Listeria* spp. on each medium. In comparison, *Listeria rocourtiae* DSM22097 and *Listeria weihenstephanensis* DSM24698 were unable to cultivate on PALCAM reference agar. Positive results were obtained with iQ-Check *Listeria* spp. while the iQ-Check *Listeria monocytogenes* tests were negative.

Significance: Currently existing methods for the detection of Listeria spp. such are suitable for the detection of the new strains *Listeria rocourtiae*, *Listeria marthii*, *Listeria weihenstephanensis* and *Listeria fleischmannii.*

P1-45 Development and Preliminary Evaluation of a Real-time PCR Assay for the Detection of *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a significant foodborne pathogen. Because outbreaks of listeriosis in humans predominantly stem from the ingestion of contaminated food, there is a need for sensitive, accurate, and rapid test methods for the detection of *L. monocytogenes* in a wide range of food and environmental samples.

Purpose: The purpose of this study was to evaluate a newly developed real-time PCR assay capable of detecting all serotypes of *Listeria monocytogenes*. Inclusivity and exclusivity testing, along with assay sensitivity, were assessed in these studies.

Methods: Cellular lysates from overnight cultures were used in these studies, with lysis carried out using a modified protocol. Cell lysates from overnight BHI cultures were used to test inclusivity at a level approximately 1-log above the limit of detection of the assay (cultures diluted to approximately 10^4 - 10^5 CFU/ml), and exclusivity (cultures at ≥ 10^8 - 10^9 CFU/ml). The sensitivity of the real-time assay was tested using cell lysates from serially diluted and enumerated cell cultures (diluted to approximately 10 CFU/ml) inoculated into a variety of food matrices.

Results: The sensitivity of the assay was shown to be ≤ $1x10^4$ CFU/ml. The assay was 100% inclusive for all the strains tested (n = 53). Exclusivity was also determined to be 100% against other *Listeria*spp. and closely related genera (n = 77).

Significance: These results demonstrate the feasibility of a new real-time PCR assay, with an alternative lysis method, for the detection of *L. monocytogenes*. This new assay allows for a more rapid time-to-result for the testing of food and environmental samples, while maintaining the simplicity, accuracy and reliability of the BAX® System method.

P1-46 An Alternative Tool for Monitoring the Presence of *Listeria* spp. in the Industrial Environment

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Introduction: Environmental monitoring is recognized as an important strategy to control *Listeria spp.* in food processing facilities. In addition to contamination at food-contact areas, the target pathogen can be found in many non-processing areas increasing the probability of the pathogen being transferred to the food products.

Purpose: The main goal was to evaluate FoodChek™ Listeria spp., an immunomagnetic lateral flow assay, as a tool for monitoring *Listeria* spp. in industrial environment.

Methods: Non-food contact surfaces (180) were artificially contaminated with *Listeria* spp., dried overnight and swabbed. Half of the samples were tested by the new assay while the remaining were tested by the USDA-FSIS cultural method. The difference between both methods was evaluated by χ^2-analysis.

The efficacy of the assay was evaluated using 48 sponge samples that were collected from non-food contact surfaces at the deli meat-slicing department. The sponges were then used in the laboratory, for sampling surfaces artificially contaminated with *Listeria*. All results were confirmed using the reference culture method.

Results: Single-step enrichment with ACTERO™ Listeria medium at 32°C for 24–28 hours was determined as the most efficient for following detection of *Listeria* spp. by the assay. For ceramic and sealed concrete surfaces, no significant differences were found in the number of positive outcomes obtained by the assay when compared to the double-step enrichment reference method MLG 8.08. However, for rubber surfaces, the assay showed significantly higher performance (χ^2 = 5.6) as compared to MLG 8.08. Neither false negatives nor false positive outcomes were documented.

All sponge samples collected at the deli meat department were correctly detected using the assay. The results were confirmed by the transfer to double enrichment step. There were no cases of contamination of the samples with wild *Listeria* spp.

Significance: The new assay presents accurate, rapid and simple alternative to standard culture methods for *Listeria* detection in industrial environment.

P1-47 Comparison of Two Selective Media and Validation of Conventional and Real-time PCR Assays for the Detection of *Listeria monocytogenes* in Food Matrices

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Introduction: *Listeria monocytogenes* is a bacterial foodborne pathogen responsible for listeriosis. Most countries have a zero-tolerance policy toward the presence of *L. monocytogenes* in ready-to-eat (RTE) foods owing to the possible severe consequences. Various methodologies, including conventional culture, molecular biological, biochemical and immunological techniques, have been implemented for the rapid and specific detection of *L. monocytogenes*. However, not all methods are well suited for routine use.

Purpose: In this study, we compared four detection methods (two standard selective media, conventional PCR and real-time PCR) most commonly used for the detection of *Listeria monocytogenes* in foods and identified false-positive colonies hindering the specific detection of *L. monocytogenes* on standard media.

Methods: *L. monocytogenes* was artificially inoculated into various food samples to generate partial positives and partial negatives. The inoculated samples were pre-enriched in *Listeria* enrichment broth followed by secondary enrichment in Fraser broth. Secondary enrichments were streaked onto Oxford and polymyxin-acriflavine-LiCl-ceftazidime-aesculin-mannitol (PALCAM) agar. After incubation, suspected colonies were biochemically identified using Vitek 2 system. In parallel, conventional PCR and real-time PCR were conducted with genomic DNA extracted from the secondary enrichment broth.

Results: Among all the methods, real-time PCR exhibited statistically excellent detection sensitivity ($P < 0.05$). Conventional culture methods performed poorly in the case of food with high levels of background microflora, generating numerous false-negative results. Although the detection of *L. monocytogenes* in fresh-cut vegetables using current culture methods was hindered only by *Listeria innocua*, various background microflora such as *Listeria innocua*, *Listeria welshimeri*, *Listeria grayi*, and *Enterococcus faecalis* appeared as presumptive positive colonies in raw beef, indicating a need to improve these methods.

Significance: We suggest that real-time PCR be used for early screening of *L. monocytogenes* in food samples, especially those with high levels of background microflora, thus complementing standard culture methodologies.

P1-48 Modification of Bolton Broth by Supplementation of Triclosan for the Isolation of *Campylobacter jejuni* and *Campylobacter coli* in Chicken Carcass Rinse

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Introduction: Triclosan has been used as an antibiotic supplement on *Yersinia* and *Pseudomonas* selective agars and has activity against a broad range of Gram-positive and most Gram-negative organisms. Innate resistance of *Campylobacter* spp., including *C. fetus* and *C. gracilis*, against triclosan has been reported. Given that its mode of action against Gram-negative bacteria differs from that of cephalosporins, triclosan has potential as a selective supplement to inhibit cefoperazone-resistant flora such as ESBL-producing *Escherichia coli* in cefoperazone-based enrichment broth used for detection of *C. jejuni* or *C. coli*.

Purpose: The aim of this study was to compare the detection ability and selectivity of triclosan-supplemented Bolton broth (T-Bolton broth) with normal broth during the isolation of *Campylobacter* spp. from whole chicken carcass rinses.

Methods: In total, 80 chickens were rinsed with 400 ml buffered peptone water. A 25-ml aliquot of rinsate was combined with 25 ml (blood-free) of 2 × Bolton broth or 2× T-Bolton broth prior to incubation at 42°C for 48h under microaerobic conditions. A loopful of each enrichment culture was then streaked onto mCCDA and incubated at 42°C for another 48h under microaerobic conditions.

Results: We compared the performance of two broths in combination with mCCDA for the isolation of *C. jejuni* or *C. coli* from chicken carcass rinse. The number of *Campylobacter*-positive samples was significantly higher in T-Bolton broth (57/80, 71.3%) than in normal Bolton broth (22/80, 27.5%) ($P < 0.05$). Furthermore, mCCDA plates prepared from T-Bolton broth samples exhibited much lower levels of contamination (3/80, 3.8%) than in those prepared from normal Bolton broth (60/80, 75.0%) ($P < 0.05$), indicating the superior selectivity of T-Bolton broth.

Significance: Relative to normal broth, the supplemented broth showed significant improvements in isolation rate and selectivity with respect to the recovery of *C. jejuni* and *C. coli* from chicken carcass rinse.

P1-49 An Independent Laboratory Evaluation of Mericon™ *Listeria* and *L. monocytogenes* Detection Kits

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Introduction: The *mericon™ Listeria species* and *Listeria monocytogenes* methods combine one of two straightforward sample preparation methods with real-time PCR detection. For either purification procedure, the resultant DNA is combined with PCR Master Mix and real-time PCR is conducted on the Rotor-Gene Q platform.

Purpose: The purpose of this evaluation was to conduct a comparison of the new method to the FDA, USDA or OMA reference methods for the detection of *Listeria* spp. *or L. monocytogenes* in selected foods and environmental surfaces as part of the AOAC-RI™ PTM validation process.

Methods: The method comparison analyzed 10 matrices: hot dogs, deli turkey, smoked salmon, mung bean sprouts, pasteurized whole milk, Gouda cheese and stainless steel, ceramic tile, plastic, and sealed concrete in an unpaired study. Matrices were co-inoculated at two levels (0.2-2 CFU/25g and 2-5 CFU/25g) with a different strain of *L. monocytogenes* and *Listeria* spp. For each matrix, Oxoid Novel Enrichment Broth (ONE Broth) plus ONE Broth Selective Supplement was validated. DNA was extracted using both the manual *mericon*™ DNA Bacteria Kit or the automated QIAsymphony *mericon*™ Bacteria Kit, analyzed by the RotorGene real-time PCR system for *Listeria* spp. or *L. monocytogenes* and compared to the appropriate reference method. Test kits were also evaluated for ruggedness, inclusivity and exclusivity.

Results: The method comparison demonstrated no statistically significant differences (POD statistical model) in the number of positive samples detected by the *mericon* candidate methods and the reference methods for all 10 matrices studied. All 50 inclusivity strains were detected. None of the 30 non-target exclusivity strains was detected. The ruggedness evaluation indicated that minor modifications in the procedure did not affect the outcome

Significance: This new method is an efficient and reliable alternative to traditional reference methods for detecting *Listeria species* and *L. monocytogenes* in selected foods and environmental samples.

P1-50 Use of the RapidChek® *Listeria* F.A.S.T. Test System to Detect Low Levels of *Listeria* Species in Composite Environmental Sponge Samples

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Introduction: Numerous listeriosis outbreaks have linked the food processing environment as a source of *Listeria monocytogenes* contamination of food. The USDA FSIS has recently published guidelines that require meat and poultry processors to test for the presence of *Listeria monocytogenes* or indicator organisms such as *Listeria* spp. in the food processing environment. Sample compositing is under consideration as a means to save food producers labor and cost while maintaining robust test performance.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® *Listeria* F.A.S.T. test system compared to the USDA-FSIS method (MLG8.09) for the detection of low levels of various *Listeria* spp. in composite environmental sponge samples.

Methods: Three strains of *Listeria* (*L. monocytogenes* ATCC 13932, *L. innocua* ATCC 33090 and *L. seeligeri* ATCC 51334) were used to spike sponges. Seventy-five composite sponge samples (5 sponges per sample) were analyzed by both methods. For each strain, 20 spiked samples (1 CFU per sample) and 5 non-spiked samples were tested. The test system composite samples were enriched in 300 ml media while USDA samples were enriched according to MLG 8.09 in 500 ml UVM and transferred to Fraser broth. All samples were confirmed for the presence of *Listeria* spp. through biochemical methods.

Results: The test system reported forty-five confirmed positive results while the MLG method reported forty-three. The 24h test system demonstrated equivalent performance as compared to the MLG method (overall χ^2= 0.24) resulting in 106% overall accuracy, 100% average sensitivity and 100% average specificity.

Significance: The test system detected low levels of *Listeria* spp. with less media volume and in a shorter timeframe (24h). This should enable food producers to quickly and efficiently identify *Listeria* spp. in a potentially-contaminated environment, with reduced time and labor inputs while maintaining test method performance.

P1-51 Comparative Validation Study to Demonstrate the Equivalence of an 8-hour Dual *Salmonella* and *E. coli* O157:H7 Enrichment for Assurance GDS® to Culture Methods for the Detection of *Salmonella* in Selected Foods and Environ

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Introduction: Many foods are commonly tested for both *Salmonella* and *Escherichia coli* O157:H7. Typically, these foods are enriched separately, increasing time and lab costs. This study proposed harmonizing these enrichments so food samples could be enriched in a single modified EHEC (mEHEC®) broth validated for use with GDS for *E. coli* O157:H7 (OMA 2005.04) in as little as 8 hours.

Purpose: To demonstrate the equivalence of GDS and new mEHEC enrichment to reference culture methods for the detection of *Salmonella* in selected foods and environmental surfaces.

Methods: Eight food matrices (cooked poultry, raw beef trim, raw ground beef, leaf lettuce, spinach, mixed greens, strawberries, and almonds) and 2 environmental surfaces (stainless steel and rubber) were included in the study. For each sample type, 20 samples were inoculated with low levels of *Salmonella*, 5 samples were inoculated with high levels of *Salmonella* and 5 uninoculated samples were included as controls. 25 g and environmental samples were enriched at a 1:10 sample to media ratio in mEHEC for 8 – 18h at 42°C. 375 g samples were enriched at a 1:5 sample to media ratio in mEHEC for 10 – 18h at 42°C. All samples were analyzed using GDS for *Salmonella* Tq and the reference culture methods, EN ISO 6579:2002. Inclusivity and exclusivity of GDS was determined by analyzing 105 strains of *Salmonella* and 30 strains of potential cross-reacting organisms.

Results: Fractional recovery of *Salmonella* was achieved for all food types tested. In total, there were valid results from 300 samples and controls. POD statistical analyses of the unmatched samples indicated that for all foods analyzed, there was no statistical difference between the GDS presumptive compared to the GDS confirmed. Furthermore, GDS method results were statistically not different than the reference culture method for the detection of *Salmonella*.

Significance: The newly harmonized mEHEC enrichment for both *Salmonella* and *E. coli* O157:H7 saves time and lowers lab testing costs for customers.

P1-52 Comparison of Testing Efficiency among Three Rapid Pathogen Detection Platforms

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Introduction: The level of scrutiny on pathogen testing within the food industry has never been higher. As a result, new pathogen detection systems have come to market with claims of improved performance and time savings. While system performance is typically validated through government or third-party agencies, studies are rarely conducted to determine true time and labour savings for a new system.

Purpose: The objective of this study was to evaluate three rapid pathogen detection systems and compare their total time to result for the detection of *Listeria* spp. in environmental samples. The following systems were compared: the DuPont™ BAX® System, the BioRad iQ-Check™, and the 3M™ Molecular Detection System.

Methods: The three systems were compared by a Canadian third-party accredited laboratory, following each system's specific method as published in the Canadian Compendium of Analytical Methods (MFLP-15 for BAX, MFLP-39 for iQ-Check, and MFLP-05 for the 3M™ Molecular Detection Assay *Listeria*). The steps in each method were grouped into five categories: media preparation, sample preparation and enrichment, software setup and lysis, assay setup, and instrument run time. Uninoculated sponges were tested on each method by the same analyst and times were recorded for each step. ANOVA analysis was used to determine significant differences.

Results: 20 runs of 50 samples were conducted for each system, totaling 1000 individual samples. The BAX method was shown to be 25 hours slower for total time-to-result than either the 3M Molecular Detection Assay *Listeria* or iQ-Check methods. The 3M Molecular Detection Assay *Listeria* was approximately 28 minutes faster than the iQ-Check for total time-to-result.

Significance: Time-to-result is an important factor to consider when choosing a rapid pathogen detection system. In a study conducted by a third-party laboratory, the 3M Molecular Detection Assay *Listeria* was shown to have a faster time-to-result than other Health Canada approved *Listeria* spp. methods

P1-53 Use of Non-proprietary Harmonized Enrichment Media for the Detection of *Escherichia coli* O157:H7, Non-O157 STEC, and *Salmonella* in 375 g Beef and Produce Samples with the Atlas® System

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Introduction: Rapid methods are necessary for the detection of pathogenic *Escherichia coli* O157:H7, non-O157 STEC, and *Salmonella* in the beef and fresh produce industry at appropriate sample sizes in accordance with industry needs. The use of a non-proprietary, harmonized enrichment media significantly streamlines the testing process for these pathogens, as well as considerably reduces cost by eliminating the need to prepare multiple enrichments.

Purpose: To validate the use of a harmonized enrichment media to detect *E. coli* O157:H7, non-O157 STEC, and *Salmonella* in artificially inoculated ground beef and romaine lettuce samples with Atlas *E. coli* and *Salmonella* assays.

Methods: 375 g test portions were inoculated with low levels of *E. coli* O157:H7, non-O157 STEC and *Salmonella* and diluted 1:5 in modified Tryptic Soy Broth + 10 g/l casamino acids (mTSB) for ground beef and Universal Pre-enrichment Broth (UPB) for romaine lettuce. All samples were enriched for 10 - 12h at 42°C, transferred into a Sample Tube and loaded onto the instrument. The instrument combines target capture, transcription-mediated amplification, and hybridization protection assay. The results of the candidate methods were compared to the FSIS and FDA reference methods.

Results: *E. coli* O157:H7, non-O157 STEC, and *Salmonella* were specifically detected after 10 - 12h enrichment in 375 g ground beef and lettuce samples. All three Atlas assays performed equally compared to the respective reference methods determined by POD analysis.

Significance: The *E. coli* O157:H7 EG2 Detection Assay, STEC EG2 Combo Detection Assay, and *Salmonella* G2 Detection Assays provide fast and highly accurate detection of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* in 375 g ground beef and lettuce samples using a non-proprietary commercially available media. The harmonized media provides a simplified testing process with significant economic and efficiency advantages along with the highly accurate results required.

P1-54 Real-time Multiplex Detection of *Escherichia coli* O157:H7 and *Salmonella* in Raw Non-intact Beef Using Housekeeping Genes as Targets

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Introduction: *Escherichia coli* O157:H7 and *Salmonella* pose a significant public health and economic burden on our society. Rapid methods are needed to detect these pathogens in perishable products before they are released into market.

Purpose: While several Real-Time Polymerase Chain Reaction (RTi-PCR) assays are commercially available, the genetic targets of these assays are not disclosed making it more difficult to interpret indeterminate results. The purpose of this study was to develop a RTi-PCR assay to detect *E. coli* O157:H7 and *Salmonella* individually, or in combination, based on housekeeping gene sequences, in raw non-intact beef.

Methods: Conserved sequences within the housekeeping genes Z3276 and *ttrC* were chosen as molecular targets for primer and probe design to amplify *E. coli* O157:H7 and *Salmonella* specific sequences, respectively. Modifications of MLG 5B.03 were performed to decrease enrichment time and provide a more efficient DNA extraction using beef trim samples. Inclusivity and exclusivity panels of at least 25 isolates were investigated with conventional and real-time PCR. Exclusivity panels included closely related strains of *E. coli* O157:H7 (*E. coli* O55) and *Salmonella* (*Proteus* and *Citrobacter).*The use of a randomly generated sequence, not naturally occurring in nature, was used as an internal amplification control.

Results: *E. coli* O157:H7 primers detected all isolates in the inclusivity panel (n = 31) and none of the isolates in the exclusivity panel (n = 27*). E. coli* O157:H7 primers detected 4 *E. coli* O157:NM. *Salmonella* primers detected all inclusivity isolates (n = 32) and detected no isolates of the exclusivity panel (n = 25). Raw, non-intact beef was inoculated with 10 CFU/g of *E. coli* O157:H7 and incubated for 10h at 42°C in pre-warmed phosphate buffered tryptic soy broth. A 2 min microwave lysis and a quicker DNA extraction resulted in a RTi-PCR Ct value of 20.22 and 19498.22 dR (2547.55 dR threshold).

Significance: The shortened enrichment and DNA extraction protocol will facilitate rapid detection of *E. coli* O157:H7 and *Salmonella* in perishable foods. The RTi-PCR assay developed here can be adapted to different real-time chemistries and performed on an open system.

P1-55 Development and Application of Selective Enrichment Broth (CES) for Simultaneous Growth of *Cronobacter sakazakii*, *Escherichia coli* O157:H7 and *Salmonella* spp

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Introduction: Detection of multi-pathogens in a single-assay platform reduces the cost and labor for testing food samples. For the effective detection of multi-pathogens, selective enrichment medium is essential to allow the concurrent growth of pathogens.

Purpose: The selective enrichment broth(CES) for simultaneous growth of *Cronobacter sakazakii*, *Escherichia coli* O157:H7, and *Salmonella* spp. was developed and applied for the detection of pathogens from agricultural produces.

Methods: 120 strains of target and non-target bacteria were inoculated and incubated in CES for 24h to investigate the spectra of bacterial growth in CES. To examine the growth kinetics of each pathogen in CES, three target pathogens were simultaneously cultured in CES in various proportions (1:1:1, 1:10:100). Finally, the performance of CES was examined by PCR assay after enriching pathogen-inoculated produce.

Results: Growth of 74.3% of non-target bacteria were inhibited in CES. In mixed-culture experiments with the three species in equal concentrations or at a 1:10:1,00 ratio, the overall growth was proportional to the initial inoculation levels. CES was able to resuscitate acid- and cold-stressed cells and recovery was comparable to that in non-selective tryptic soy broth. Moreover, target pathogens were efficiently detected by PCR assay after enrichment in CES.

Significance: CES was demonstrated to be a promising new multiplex selective enrichment broth for the detection of the three foodborne pathogens by biochemical or nucleic acid-based methods.

P1-56 A Robust Multiplex Real-time PCR Method for Simultaneous Detection of *Salmonella* spp., *Escherichia coli* O157 and *Listeria monocytogenes* in Fresh Fruits and Vegetables

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Introduction: On average, about 48 million people per year in the US are affected by foodborne diseases. A major portion of these illnesses are caused by *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Hence, it is important to identify these pathogens in contaminated foods so that they can be eliminated from the marketplace, and thereby reducing the incidence of foodborne diseases. At present, there is no good method available for the simultaneous detection of these organisms in foods regulated by FDA.

Purpose: This project aims to optimize, and adapt a multiplex real-time PCR method, originally developed for meats at the USDA, to simultaneously detect the presence of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes* in salads, sprouts, cantaloupes, strawberries, mangoes and peanut butter.

Methods: Food samples were spiked with 5-25 CFU/25g of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* stomached and incubated for 2 hours at 37°C in Buffered Listeria Enrichment Broth, followed by the addition of nalidixic acid, fosfomycin, cycloheximide and acriflavine and grown overnight. The enriched culture was centrifuged and the pellet was used for the extraction of genomic DNA (Qiagen). The DNAs were used to carry out the multiplex real-time PCR with primers and TaqMan probes specifically targeting *invA* (*Salmonella*), *rfbE* (*E. coli* O157), *hlyA* (*L. monocytogenes),* and an internal amplification control.

Results: All three gene targets of the tested pathogens were detected in the spiked salads, sprouts, cantaloupes, strawberries, mangoes, and peanut butter, with a sensitivity of 5-25 CFU/25g. The gene targets were not detectable in the non-spiked controls. Extraction of template DNA from peanut butter was time consuming because of its viscosity.

Significance: This method can be applied to detecting pathogens from the food matrices involved in outbreaks, thus allowing the FDA to take swift action and prevent the spread of an outbreak.

P1-57 Chitosan-based Antibacterial Microparticles: Mode of Action in the Control of Pathogens Associated with Fresh Produce

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Developing Scientist Competition

Introduction: Novel non-thermal methods are in demand to improve the microbial safety of fresh and fresh-cut fruits and vegetables. Studies on the antimicrobial properties of chitosan suggest high potential of chitosan microparticle application in pathogen control, microbial population reduction, and delivering antimicrobial materials.

Purpose: The purpose of this project was to develop a chitosan-based, antimicrobial-coupled microparticle to be applied through immersion, drench or spray on fresh and fresh-cut fruits and vegetables. Studies were conducted to determine antagonistic or additive effect when combining chitosan microparticles with other antimicrobials.

Methods: Plain chitosan microparticles (~270nm) were produced by adding sodium tripolyphosphate (TPP) to chitosan solution. The mixture was centrifuged and microparticles were resuspended in water and checked for size and zeta-potential. Antimicrobial activity was tested at neutral and acidic pH in TSB against Gram-positive and Gram-negative bacteria. Studies were performed in water at pH 6 using particles and chitosan solution. A panel of antimicrobials commonly used in food (e.g., EDDS, nisin and lauric arginate) were also evaluated and, based on their Minimal Inhibitory Concentration (MIC), were tested in tandem with chitosan microparticles.

Results: The MIC of the particles was found to be > 2mg/ml at neutral pH for most strains, at lower pH the MIC was reduced for G(+) bacteria; e.g. *L. monocytogenes* (~0.9 mg/ml). Chitosan solution and particles elicited an effect on the survival of G(+) bacteria within 30 min of exposure, and both inhibited the growth of *L. monocytogenes* and *Salmonella* over a 24h period.

Significance: The microparticles can be used independently or in conjunction with sanitizing wash to inactivate foodborne pathogens on fresh produce immediately prior to packaging, providing a food-safe and low-cost alternative to control foodborne pathogens.

P1-58 Sanitizer Efficacy Protocol Using a Dried Microbial Model

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Introduction: By employing a realistic application model we tested the efficacy of various sanitizer types. The method uses a dried microbial film of known challenge & a set clone of replicated challenge strain using a membrane platform test system that is fully recoverable for microbial survivors.

Purpose: This study was to evaluate the efficacy of quaternary ammonia, peroxyacetic acid, sodium hypochlorite, and chlorine dioxide based sanitizers against various microbial strains utilizing a dried microbial film method to simulate realistic plant conditions and actual sanitizer applications on contaminated surfaces in operational processing plants. Each strain was dried onto Hydrophobic Grid Membrane Filters [HGMF], and employed a modified disinfectant efficacy test to be used with various biocidal sanitizers. The microbial challenge strains used were both vegetative and sporeforming bacterial pathogens, and spoilage fungi & yeasts.

Methods: The microbial strains; *S.* Typhimurium, *S. aureus*, *L. monocytogenes, B. cereus, P. aeruginosa, E. coli* 0157:H7, *A. Niger, S. cerevisiae, P. notatum* were all grown in fresh liquid culture, and suspended in 0.1% Peptone. These suspensions were diluted at a challenge range of 6 - 8 log cycles for bacteria, 5 – 6 log cycles for fungi. The suspensions were uniformly filtered and dried onto HGMF filters [IsoGrids, Neogen Corp., MI]. The inoculated filters were then exposed to the various sanitizers at set concentration levels. The sanitizers were fully neutralized with Letheen broth after a contact time dependent on the microbial strain. Upon neutralization the HGMF filters were plated onto nutrient agar obtaining complete recovery of the remaining viable cells. These exposed filters were then compared against positive control filter titers to determine log reductions.

Results: Chlorine dioxide showed the most bacterial reduction overall ranging from 4-7 logs CFU/ ml. The quaternary ammonia sanitizers showed the best per concentration at preventing fungal spore germination 4-6 log CFU/ml compared to chlorine dioxide range of 4-5 log CFU/ml. Sodium hypochlorite, peroxyacetic acid sanitizers all show to be efficacious on certain strains tested but not as broad of a reduction shown by chlorine dioxide and quaternary ammonia based sanitizers.

Significance: This real world test model enables the food processor sanitarian & food safety personnel to determine which biocides/sanitizers best fit their needs in terms of the pathogens/spoilage microbes of concern on dried films of microbes which is the most predominate challenge

environment for the sanitizers in question. By using a set stock suspension of the test microbe you create the same genetic clones for your sanitizer studies over a short but workable time frame of 1-2 weeks. This provides data between replicates that has very slight experimental variability.

P1-59 Development and Application of Novel SNP-based Serotyping Assays in Targeting *Salmonella enterica* within the Poultry Production and Processing Continuum

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Introduction: *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S.* Enteritidis) is the leading cause of salmonellosis worldwide. While some *S. enterica* serotypes are specific to birds, many represent human zoonotic pathogens, thus their presence and survival throughout the continuum of poultry production environments is of vital concern to the poultry industry.

Purpose: It is essential to rapidly type the various *Salmonella* to determine potential food safety-related issues, especially those that occur within the serotypes linked to disease. A novel approach to molecular serotyping of poultry-related *Salmonella* would be to type them based on single nucleotide polymorphisms (SNPs) within the genetic code of these diverse serotypes.

Methods: Primers were designed to target ~ 300-bp regions of both a virulence-associated (*cyaA*) and non-virulence (*ushA*) genes that contained descriptive SNPs for 4–5 serotypes (3 Enteritidis, Typhi, Typhimurium, Heidelberg, Fetus and Kentucky). Hybridization probes targeted these descriptive SNPs and Luminex MagPlex hybridization protocols were developed and optimized. Both assays were validated using strains recovered from environmental samples from throughout the production continuum.

Results: Both assays were found to be highly specific and sensitive towards their respective SNP targets, even when testing samples containing all target SNPs for a given assay. For most environmental isolates tested, the assays were able to effectively pathotype different *S.* Enteritidis strains and serotype different *S. enterica*, as well as quickly differentiating between Kentucky and Enteritidis recovered as part of a poultry challenge study.

Significance: These data highlight the potential of SNP-based assays to rapidly serotype poultry-related *Salmonella enterica*. Expanding this method to include additional serotype-specific SNPs, or applying it directly to isolates recovered from challenge studies will allow us to not only rapidly determine which *Salmonella* are recovered, but also which environmental parameters control the survival of the serotypes within different poultry models.

P1-60 Viability PCR: An Evaluation of Propidium Monoazide for Live/Dead Differentiation of Microorganisms

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Introduction: Nucleic acid detection methods such as real-time PCR (rt-PCR) provide fast and powerful tools to analyze samples for the presence of potentially harmful microbes, but also hold the risk of false positives by detecting nucleic acid from harmless dead cells. Viability PCR utilizes the DNA-masking compound propidium monoazide (PMA) which is able to enter dead and membrane-compromised cells, modifying the DNA on photo-activation, resulting in a strong inhibition of PCR amplification.

Purpose: The aim of this study was to evaluate the performance of viability PCR centering on PMA and a new illumination device with several pathogens and matrices

Methods: Defined mixtures of live-dead pathogens were prepared (*Salmonella* spp. and *Listeria* spp.) and treated with/without PMA. Pathogen mixtures were illuminated at a specific wavelength to irreversibly bind the reagent to dead cell DNA, rendering it no longer PCR visible. Efficient suppression of amplification of such modified DNA allows preferential rt-PCR detection of live cells. The performance in turbid and colored mixtures was also studied.

Results: The sensitivity of the method is determined with titrations of different live/dead pathogen ratios (100% dead to 100% live). The rt-PCR performance data from these cell mixtures with or without PMA treatment demonstrates the extent of the masking effect of PMA on dead cells. Dead cells treated with PMA show a ~15 higher Ct value their non-PMA treated counterpart ($X^2 < 0.05$). The data using turbid/colored matrices demonstrates that the PMA activating light is sufficient to penetrate such samples, to lead to successful DNA intercalation and allow live/dead detection.

Significance: Live/dead differentiation can play an important role in procedures such as hygiene monitoring (success of decontamination processes), water testing (distinguishing between live and dead legionella for regulatory compliance) and human diagnostics (monitoring medication efficiency in pathogen killing).

P1-61 Single Laboratory Validation of a *Vibrio* TaqMan qPCR 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. For detection of vibrios, 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**.** This study compares the Life Technologies multiplex *Vibrio* qPCR assay with the BAM procedure for identification of *Vibrio* isolates.

Purpose: To evaluate real time PCR for rapid identification of vibrios.

Methods: *Vibrio* cultures were grown in APW overnight at 35°C, streaked onto selective agars and confirmed biochemically as Vc, Vv, or Vp using methods described in the BAM including API20E biochemical identification and conventional PCR for Vc, Vv, and Vp as outlined in the BAM. For the *Vibrio* TaqMan Assay*, Vibrio* isolates were grown on T_1N_0 agar (Vc) or T_1N_3 agar (Vp and Vv). To prepare template, a well isolated colony was suspended in saline, boiled for 10 min., centrifuged 15,000 x g for 3 min. and the supernatant was used for vibrio detection. Template was diluted (1:100) for multiplex qPCR detection of Vc, Vv, and Vp as described by the manufacturer.

Results: The *Vibrio* TaqMan assay correctly identified 50/52 Vc isolates while the BAM procedure (API 20E) identified all 52. For Vv, 50/51 isolates were positive by the *Vibrio* qPCR assay, 45/51 by API20E and 50/51 by the FDA BAM PCR. Both the *Vibrio* qPCR assay and the BAM PCR confirmed 50/50 isolates tested while the API20E identified 49/50 Vp isolates correctly. Of the 45 near-neighbor *Vibrio* isolates, as identified by API20E, none were detected by the *Vibrio* qPCR assay.

Significance: The results presented here demonstrate the Life Technologies *Vibrio* TaqMan assay is a reliable and rapid alternative to the BAM methods for identification of Vc, Vv, and Vp isolates.

P1-62 Comparison of Dehydrated Film Media for the Quantitative Enumeration of Yeasts and Molds

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Introduction: Yeast and Molds are ubiquitous food spoilage agents that can grow at a wide range of temperatures, pH values and more importantly at reduced water activities (a_w) thus having the potential for substantial economic losses to the food industry. Recently a variety of alternate microbiological technologies have become available which provide the end user with new offerings having a claimed 2-3 day read out time along with results that are available in the more traditional 5-7 day time frame.

Purpose: This study was conducted to evaluate the comparative performance of 3 different dehydrated film media and reference methodology as described in FDA/BAM and ISO 21527:2008 (parts 1 and 2).

Methods: The method comparison was conducted using a variety of naturally contaminated food matrices (n = 65) with the dehydrated film methods and the reference method (FDA/BAM and ISO21527:2008, parts A and B). Each food sample was serially diluted in 0.1% peptone water. The dehydrated film media were hydrated with 1ml of the sample and 100 micro-liters were spread plated on DRBC or DG-18 media in duplicate.

Results: Statistical comparisons between the various dehydrated film methods at the prescribed readout times and the reference DRBC and DG-18 at 5 days demonstrated significant differences in *P* values (< 0.05) depending on the method. Additional qualitative differences in the appearance and handling of the various dehydrated film media were also observed.

Significance: Rapid test results are increasingly becoming more important for food processors. Developing tests with faster time to results without sacrificing performance is the ultimate goal. As newer technologies providing alternate methods become readily available, understanding the performance, recovery and handling across the various methods provides food microbiology laboratories with valuable information that will help informed decision making towards the method of choice.

P1-63 Rapid Resin-based Method for Concentration of Rotavirus, Hepatitis A Virus and Adenovirus 40 from Tap Water

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Introduction: Detection of low levels of viral contaminants in water represents a food safety challenge, due to the lack of rapid, simple and inexpensive methods for concentration of targets before detection. Currently, most virus concentration methods are based on adsorption of viruses to filters by electrostatic interactions, followed by elution of viral particles and nucleic acid isolation for further molecular detection. However, these systems display several shortcomings, including clogging, need for large volumes of eluent, low recovery efficiency and low sensitivity.

Purpose: Evaluate the performance of a newly developed resin-based concentration method to detect low concentrations of hepatitis A virus (HAV), adenovirus 40 (HAdV-40) and rotavirus (RV) in tap water.

Methods: Ten-liter tap water samples (n = 3) inoculated with different concentrations (10 to 10,000 $TCID_{50}$/10 l) of human adenovirus 40, hepatitis A virus and rotavirus were incubated for 120 minutes with 0.5g of anion exchange resin, followed by nucleic acids isolation directly from the resin. Nucleic acids isolated from water before concentration and from the resin after concentration were analyzed by real time PCR or real time reverse transcriptase PCR, using commercially available detection kits (Ceeram®).

Results: Detection of enteric viruses from the resin after concentration was significantly ($P < 0.001$) improved compared to detection from water samples (average ΔCt = 12.1 for AdV-40, 4.3 for HAV and 7.0 for RV). The lowest virus concentration detected using resin-based concentration was 10 $TCID_{50}$/10 l for HAdV-40 and HAV, and 100 $TCID_{50}$/10 l for RV. The resin-based virus concentration method compares favorably to other methodologies, considering that it displayed similar detection limits, but it was easier to perform and more cost effective.

Significance: The resin-based method rapidly and successfully concentrates different enteric viruses from water samples, ultimately enhancing molecular detection sensitivity.

P1-64 Detection of Viable Murine Norovirus Using Plaque Assay and Propidium Monoazide Real-time Reverse Transcription-polymerase Chain Reaction

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Developing Scientist Competition

Introduction: Norovirus is the most common cause of gastroenteritis worldwide. Lacking of virus culture system, it is hard to determine viability of norovirus only by reverse transcription-polymerase chain reaction (RT-PCR) or real-time RT-PCR.

Purpose: The aim of this study was to investigate the detection of viable murine norovirus (MNV) by the combination of propidium monoazide (PMA) treatment and real-time RT-PCR.

Methods: Five log PFU/ml MNV-1 was inactivated by the heat treatment at 20°C, 60°C, 70°C, 80°C, and 100°C in water bath for 1 min. Plaque assay, real-time RT-PCR, and PMA-combined real-time RT-PCR was performed with heat-treated MNV.

Results: The titer of MNV was reduced 0.52, 1.00, and 2.00 log PFU/ml at heat-inactivated MNV at 60°C, 70°C, and 80°C condition, respectively. MNV were not detected at 100°C condition by plaque assay. The relative quantity values of MNV reduced 0.10 log, 0.88 log, 1.09 log, and 1.55 log at 60°C, 70°C, 80°C, and 100°C condition, respectively, by real-time RT-PCR. However, in PMA-combined real-time RT-PCR group, the relative quantity values reduced 0.47 log, 1.93 log, 2.80 log, and 3.89 log at 60°C, 70°C, 80°C, and 100°C condition, respectively.

Significance: PMA-combined real-time RT-PCR was correlated with plaque assay to detect viable MNV.

P1-65 Enhanced Reverse Transcription Polymerase Chain Reaction Enzyme-linked Immunosorbent Assay (RT-PCR-ELISA) for Simultaneous Detection of Genogroup I and Genogroup II Noroviruses

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Introduction: Noroviruses (NoVs) were identified as one of the major foodborne viruses which cause acute viral gastroenteritis. NoVs are transmitted via fecal-oral route and mainly infected through contaminated foods and water. Although various PCR assays to detect NoVs were developed not only in contaminated water and foods but also in stools, RT-PCR-ELISA which combined RT-PCR and ELISA technique does not applied for NoVs detection.

Purpose: The purpose of this study was to develop a specific and sensitive RT-PCR-ELISA for simultaneous detection of Genogroup I (GI) and Genogroup II (GII) noroviruses (NoVs) using universal probe.

Methods: Primers and probes were designed at ORF1 and ORF2 region of GI and GII NoVs. Universal probe was combined with each GI and GII NoVs probe. RT-PCR-ELISA for detection of NoVs was performed following semi-nested reverse transcription-polymerase chain reaction (semi-nested RT-PCR). DIG-labeled DNA fragments were hybridized with biotinylated universal probe at 37°C. After the conjugation with anti-digoxigenin-peroxidase antibody, ABTS colorized the positive products. Optical absorbance was measured at 405 nm.

Results: RT-PCR-ELISA using universal probe for the detection of NoVs could simultaneously detect GI and GII NoVs. RT-PCR-ELISA could detect by at least 100 copy number/µl GI and GII NoVs from human stool specimens. Compared with semi-nested RT-PCR and real-time RT-PCR, the sensitivity of RT-PCR-ELISA was comparable with real-time RT-PCR. In specificity test, RT-PCR-ELISA detected only GI and GII NoVs except hepatitis A virus, hepatitis E virus, rotavirus, adenovirus and murine norovirus.

Significance: With high sensitivity and specificity, RT-PCR-ELISA with universal probes could detect different genogroups of NoVs, simultaneously.

P1-66 Use of Kidney Inhibition Swab Test to Evaluate Antimicrobial Residues in Pork Kidney from a Market Survey in Fargo, North Dakota

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Introduction: The USDA-Food Safety and Inspection Service (FSIS) recently changed the on-site antimicrobial screening method to the Kidney Inhibition Swab (KIS™) test to be used at all livestock slaughter establishments. Although the newly adapted screening method can detect different classes of antibiotics, the test is most sensitive towards penicillin. Penicillin is one of the most commonly found tissue residue violations in annual USDA-FSIS surveys of pork. Previously, our study indicated that sows treated with label dose of penicillin G and slaughtered at the proper labeled-indicated withdrawal day tested positive when kidney was monitored with the KIS™ test.

Purpose: The purpose of this study was to determine the extent of antimicrobial residues detected by the KIS™ test in pork kidney obtained from local markets.

Methods: Pork kidneys were purchased from four grocery stores including two ethnic specialty stores every 2-4 weeks to ensure that the kidneys obtained were from different lots. Although kidneys were obtained from local grocery stores, the pork samples were from suppliers located throughout the mid-western region of North America. The KIS™ test were performed and color changes of the tests were recorded after prescribed incubation time at 64 ± 2 °C. A purple/blue color indicated antibiotics were present in the samples while yellow/green color indicated no/low antibiotics present in the samples. A "caution" indicates a sample contains antibiotics below the "positive" threshold.

Results: Two samples returned "caution" from our market survey. One "positive" sample was identified, but was not confirmed with the follow up tests.

Significance: Our findings indicate that pork kidney in the market for human consumption contained low/no antibiotics using the newly adapted USDA-FSIS antimicrobial screening test.

P1-67 Meat Species Identification Using PCR-RFLP and Native Capillary Electrophoresis System

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Introduction: For consumers protection it is necessary to develop effective methods able to verify species composition of different food products. These methods mostly rely on DNA analysis using PCR because it allows unequivocal species identification from raw as well as processed foods. The DNA fragment obtained by amplification of a specific gene is digested with restriction enzymes (PCR-RFLP) to discriminate the species. Usually, a region of the mitochondrial genome, such as tRNAGlu/cytochrome b, is amplified, followed by digestion with a number of restriction endonucleases sufficient for species identification.

Purpose: The purpose of this study was to optimize and validate an efficient PCR-RFLP protocol for meat species identification using automated detection and data analysis of the QIAxcel Advanced system.

Methods: After meat homogenization and lysis, DNA purification was performed using QIAsymphony and Qiasymphony DSP DNA mini kit. Following PCR, the resulting product of 359 bp was digested with four restriction enzymes: AluI, HaeIII, HinfI and RsaI. The digests were analyzed by native capillary electrophoresis and the results were interpreted utilizing a programmed Excel sheet.

Results: Raw meat was used, as well as the meat processed by cooking, freezing, cooking and smoking, dehydration and sterilization. The method was tested on 108 reference samples containing either only one meat (80) or mixed meat species (28). For validation, the samples were analyzed by real-time PCR and the results were compared to the data obtained by PCR- RFLP following the analysis by QIAxcel ScreenGel software. The two methods gave 100% congruent results.

Significance: Using the native and automated capillary electrophoresis significantly reduces the analysis time and minimizes the potential procedural errors, thus providing reliable and fast method for meat species identification, especially for large-scale analyses encountered in the routine food control of meat and meat products.

P1-68 Evolution of Microbiological Analytical Methods for Dairy Industry Needs

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Introduction: Fermenting microorganisms play a pivotal role in the development of physicochemical and sensory properties of food products but also contribute to product safety by limiting the growth of pathogenic and spoilage microorganisms. Therefore, evaluation of cell viability is of great importance for the fermented food industry in general, and more specifically for the dairy sector. Traditionally, culture-based methods have been used to enumerate microbial populations in dairy products. Recent developments in molecular methods now enable faster and more sensitive analyses than classical microbiology procedures. These molecular tools allow a detailed characterization of cell physiological states and bacterial fitness and thus, offer new perspectives to integration of microbial physiology monitoring to improve industrial processes.

Purpose: Review of existing methods described to enumerate and characterize physiological state of technological microbiota in dairy products. Discussion of current deficiencies related to specific needs of the dairy industry for fast, efficient, reliable and standardized methods.

Methods: In addition to a global Internet search, a total of four international databases were screened for journal articles, books, patents, conferences and symposia proceedings in the field of food science, food industry, life science and biomedical information (FSTA®, BIOSIS® Preview, Medline®, Foodline®).

Results: Strikingly, the use of chromogenic media has been developed for the analysis of pathogenic and spoilage bacteria and validated and normalized methods are available, but nothing of the kind was set up for technological microbiota. Recent studies show that PCR-based methods, flow cytometry and omics technologies show interesting analytical potentialities to quantify fermenting microbes and probiotics in dairy products. However, they still suffer from a lack of validation and standardization for quality control analyses, as reflected by the absence of performance studies and official international standards.

Significance: While standard methods have been developed and validated for foodborne pathogens, lactic acid bacteria and probiotics seem to be the poor relations of the diagnosis industry and method standardization. This is surprising when considering the amount of quality controls performed by the dairy industry.

P1-69 *Leuconostoc mesenteroides* subsp. *mesenteroides*: Bacteriocinogenic Strain Isolated from Brazilian Water-buffalo Mozzarella Cheese

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Introduction: The exploration of naturally occurring antimicrobials in food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern about microbial resistance toward conventional preservatives.

Purpose: To purify and characterize the antimicrobial protein produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55.

Methods: The production of bacteriocin was studied at 25°C and 30°C. Influence of enzymes, detergents, pH and temperature on bacteriocin was evaluated. Bacteriocin effect on *Listeria* spp. and *Enterococcus* spp. and other foodborne pathogens was determined. The bacteriocin was purified using affinity column and reversed-phase chromatography. Molecular mass of the expressed bacteriocins have been determined by mass spectrometry. DNA from strain SJRP55 have been evaluated by PCR with primers targeting mesenterocin B105 and Y105, leucocins K, A, B and A-TA33a.

Results: Cell-free supernatant of strain SJRP55 showed inhibition activity against *Listeria* spp. and *Enterococcus* spp., but not affected *Lactobacillus* spp. Antimicrobial compound was resistant to high temperatures, low pH, and chemical compounds; was not affected by α-amylase, lipase and catalase, confirming its proteinaceous nature. The optimal temperature for bacteriocin production was 25°C. Bacteriocin showed a bacteriostatic effect against several *Listeria* species and *Enterococcus faecalis*. Mass spectrometry and amino acids analyses showed that the bacteriocin produced by strain SJRP55 was similar to mesenterocin Y105 and mesenterocin B105. PCR reactions generated positive results for mesenterocin Y105 and B105 genes and after sequencing, a 100% homology have been recorded.

Significance: The bacteriocins showed high similarity to other bacteriocins produced by *Leuconostoc* species. Different environmental matrices, conditions and geographical regions seem do not affect the genes encoding bacteriocins. Probably these genes are very well conserved during the evolution of *Leuconostoc* genus. The bacteriocin produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 showed potential as a biopreservative to be applied as a tool of a system of multiple preservative principles.

P1-70 Evaluation of the 3M™ Microbial Luminescence System (MLS II) for Microbial Detection in Flavored UHT Milk

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Introduction: The 3M™ Microbial Luminescence System (MLS II) is a rapid alternative to traditional microbiological assessment of UHT products. The 3M MLS II utilizes ATP bioluminescence to detect microbial ATP which can be used as a marker for biological contamination. It enables the release of commercially sterile products after a 48 or 72 h pre-incubation period and a 26 min assay

Purpose: To compare the performance of the 3M MLS II for the release of UHT products with traditional microbial method and pH measurement

Methods: Three flavors of whole fluid milk (UHT processed & aseptically filled) were inoculated with four microorganisms (*B. subtilis*, *E. faecalis*, *P. aeruginosa*, *B. megaterium*) at each of 3 inoculation levels (low, < 10 CFU/brick; high, 10-50 CFU/brick; control, no inoculation). Samples (n = 30) were analyzed for each flavor-microorganism combination following incubation for 72h at 30°C, using the MLS, streaking on nutritive agar and pH measurement

Results: For all flavors analyzed, MLS correctly detected 100% of samples with both high and low levels of inoculums of *B. subtilis* and *P. aeruginosa*, using streaking on nutritive agar as a comparison. At both high and low levels of *E. faecalis*, MLS correctly detected 71-100% of samples tested, using streaking on nutritive agar as a comparison. Interestingly, the pH method, widely considered to be an acceptable means of determining UHT sterility, correctly detected inoculated UHT product for 6-26% of those samples tested; only chocolate UHT milk with low-level *E. faecalis* was correctly detected for all samples. These results demonstrate that, particularly for lactose non-fermenting organisms, MLS provides a more accurate means of detecting contamination of UHT dairy products

Significance: For the UHT flavored whole milk (strawberry, cookies and cream, chocolate), the MLS demonstrated comparable results to the reference methods for the rapid detection of contaminated UHT bricks in as little as 72 hours of incubation.

P1-71 Development and Inter-laboratory Validation of Aflatoxin M1 Analysis in Bovine Milk

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Introduction: Aflatoxins are of major concern to the dairy industry as ingestion of aflatoxin B1 contaminated feed by dairy cattle can result in milk containing aflatoxin M1. Validated method for quantitation of aflatoxin M1 in milk is important.

Purpose: To develop a simple sample preparation protocol together with a LC-MS/MS method for quantitation of aflatoxin M1 in bovine milk to meet both EU (50 ppt) and U.S. (500 ppt) action levels and to evaluate the performance through an inter-laboratory study.

Methods: A simple extraction procedure based on a QuEChERS-like protocol followed by protein precipitation and ultrasonication prior to LC-MS/MS analysis was developed, validated and recommended to use for a proficiency testing program. A total of eight blind coded bovine milk samples, six spiked with aflatoxin M1 in the range of 200 to 750 ppt and two unspiked samples were shipped to 37 Food Emergency Response Network (FERN) laboratories. The results from ten laboratories that used the developed method were statistically analyzed according to the internationally harmonized protocol ISO 13528:2005. The mean values and reproducibility for each sample obtained by the ten laboratories were compared to the corresponding consensus values obtained from all participating laboratories reported quantitative results.

Results: The mean concentrations of aflatoxin M1 obtained using the developed method (n = 10) were 212 ± 85, 299 ± 55, 412 ± 135, 509 ± 139, 754 ± 131ppt compared to the consensus values (n = 25) of 216 ± 84, 302 ± 84, 415 ± 183, 503 ± 175 and 739 ± 174 ppt for the spiking level of 200, 300, 400, 500 and 750 ppt, respectively. The aflatoxin M1 concentrations obtained using the developed method were not significantly different from the corresponding consensus values ($P > 0.05$).

Significance: These data showed that the developed method could be used for routine and accurate monitoring of aflatoxin M1 in bovine milk.

P1-72 A New Rapid Quantitative Method for Detection of Aerobic Plate Count from Environmental Hygiene Swabs

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Introduction: There is increased emphasis in food production facilities in maintaining and improving hygiene standards while also reducing downtime. This has prompted the development for rapid methods that would provide results within 8-12 hours (production cycle) such that corrective action can be taken and product can be released in a timely manner. Some technologies (ATP bioluminescence) meet the burden of generating contamination results in a shorter time. They do not correlate effectively to bacterial load. It has been demonstrated that having an environmental swabbing program in place reduces the chance of a foodborne outbreak.

Purpose: This study was conducted to evaluate a new oxygen-sensing method for the quantitative detection of aerobic bacteria from inoculated stainless steel surfaces with environmental hygiene swabs compared to the standard reference method.

Methods: A cocktail containing 0.1 ml of overnight cultures of *Escherichia coli*, *Enterobacter faecium*, *Salmonella* Enteritidis, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was used to inoculate stainless steel surface with 100 µl of a 10^2, 10^4 and 10^6 dilution. The surfaces and dilutions were conducted in triplicate. The inoculated surfaces were allowed to dry for 18h. They were then swabbed with both a cotton swab and a nylon swab. The swabs were placed into 15 ml sensor vial and 10 ml of tryptic soy broth (TSB) was added. The vials were then placed in the system. Concurrent plate counts were performed for quantitative comparison.

Results: Two separate trials were run with correlation coefficients of plate counts to time-of-results (oxygen depletion) for the cotton swabs were 0.96 and the nylon swab were 0.84. Results for the oxygen-sensing system showed a typical time-to-result of less than 12 hours at a bacterial load of 10 CFU per swab compared to 48h for the plate count method.

Significance: This new oxygen-sensing method would provide both quantitative and rapid (within a production cycle) results in determining the effectiveness of the sanitation program in a food facility. In addition, it also allows the release of product in a less than one day versus waiting for micro results which take 24-48 hours.

P1-73 ISO 11133 – Evaluation of Microbiological Performance Testing in Quality Control of Culture Media

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Introduction: The International Standard ISO 11133 "Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media" is currently under revision and is planned to be published mid 2014. The revised standard ISO FDIS 11133 describes all methods for the routine control of culture media. It specifies the requirements and acceptance criteria for the performance testing for the most culture media given by International Standards for food and water microbiology for the lab user and manufacturers.

Purpose: The purposes of this current study were to perform and evaluate the performance testing of culture media including the acceptance criteria given by the revised standard ISO FDIS 11133:2013.

Methods: Culture media for pre-enrichment, selective enrichment and isolation were tested according to the methods given by ISO FDIS 11133:2013. Test strains and inocula were prepared from fresh cultures in Tryptic Soy broth and diluted to the desired number of organisms for inoculation: for testing on productivity 10-100 CFU and for selectivity ≥ 10^4 CFU per tube or plate. Performance evaluation and interpretation of the results followed the specifications given by the Standard.

Results: Culture media for pre-enrichment (Buffered Peptone Water), selective enrichment (Fraser Broth) and solid isolation media (Baird Parker agar, *Listeria* agar acc. to Agosti and Ottaviani, Plate Count agar) showed on a minimum of 3 production batches the required criteria for productivity, selectivity and specificity (were applicable). Quantitative productivity tests met the required performance criteria *PR* ≥0,5 for the selective media (Baird-Parker agar: 2 strains, *Listeria* agar: 2 strains) and *PR*≥0,7 for non-selective media (Plate Count agar: 4 strains).

Significance: For the tested media the results of this study indicate the applicability of the methods and criteria for the performance testing of culture media as given in the revised International Standard ISO FDIS 11133:2013.

P1-74 Development and Validation of a Rapid Test Kit for Detection of Pork Meat Residues

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Introduction: A recent investigation into meat adulteration revealed that many foods advertised as containing beef were found to contain undeclared pork residues. Whereas consumption of pork is not harmful, certain ethnic and religious practices forbid the consumption of pork meat. Moreover, undeclared pork meat can present a health risk if consumed in an undercooked state. In an effort to safeguard the public from inadvertent exposure to pork meat, we developed a rapid and highly specific test kit capable of detecting trace levels of pork meat residues.

Purpose: To develop and validate a highly specific detection kit that can rapidly identify pork meat residues down to 0.01% contamination in xenogenic meat sources.

Methods: Polyclonal antibodies against pork serum albumin (PSA) were raised in goats and purified on a PSA-affinity column. The purified antibodies were then used to develop a flow immunochromatographic assay configured with a procedural control line, a test line, and an overload line. A sample extraction buffer and a running buffer were developed for optimal performance. The ensuing kit and test method were validated for specificity and dynamic range. Method concordance was assessed using a commercial ELISA (ELISA-TEK) kit and results were confirmed using a validated meat authentication PCR-based method (IEH).

Results: The MEI/IEH pork meat lateral flow test demonstrated a range of detection of 0.01% to 100% spiked pork meat (raw or cooked meat into beef meat). Specificity analysis revealed no cross-reactivity with serum albumins or meats derived from chicken, turkey, horse, beef, lamb, and goat. The assay was more sensitive than the commercial ELISA kit and required considerably less time than the PCR and ELISA methods.

Significance: The development of a highly specific test method capable of detecting trace amounts of horse meat in 30 min should aid the Food Safety Authorities in their continued efforts to monitor for pork meat adulteration in the food chain. Furthermore, the facile operation of this kit should enable religious parties the opportunity to independently perform meat authentication testing.

P1-75 Development and Validation of a Rapid Qualitative Test Kit for Detection of Horse Meat Residues

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Introduction: A recent European investigation into meat adulteration revealed that many foods advertised as containing beef were found to contain undeclared horse meat. Whereas consumption of horse meat is not inherently harmful, introduction of undeclared horse meat into the food supply chain significantly increases the risk of human exposure to deleterious veterinary drugs and calls into question the safety practices of several major food manufactures. In addition, consumption of horse meat in the UK and USA is verboten, as underscored by the public response that ensued following the disclosure of this scandal.

Purpose: To develop and validate a highly specific detection kit that can rapidly identify horse meat residues down to 0.01% contamination in xenogenic raw meat sources.

Methods: Polyclonal antibodies against horse serum albumin (HSA) were raised in goats and purified on an HSA-affinity column. The purified antibodies were used to develop a lateral flow immunochromatographic assay configured with a procedural control line, a test line, and an overload line. A sample extraction buffer and a running buffer were developed for optimal performance. The ensuing kit and test method were validated for specificity and dynamic range. Method concordance was assessed using a commercial ELISA (ELISA-TEK) kit and an authentication PCR-based method (IEH).

Results: The MEI/IEH horse meat lateral flow test demonstrated a range of detection of 0.01% to 100% spiked horse meat (raw or cooked into beef meat). Specificity analysis revealed no cross-reactivity with serum albumins or meats derived from chicken, turkey, pork, beef, lamb, and goat. The assay was more sensitive than the commercial ELISA kit and required considerably less time to perform than the PCR and ELISA methods.

Significance: The development of a highly specific test method capable of detecting trace amounts of horse meat in 30 min should aid Food Safety Authorities in their continued efforts to monitor for horse meat adulteration.

P1-76 Development and Validation of a Novel Gluten Detection Lateral-flow Device

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Introduction: To address growing public health concerns regarding gluten intolerance, the FDA has established regulatory threshold limits for gluten content in foods labeled "gluten-free." Implementation of these labeling laws requires antibody based methodologies that can accurately quantify gluten levels. To address the growing need for rapid and accurate detection of gluten residues, we developed a Lateral Flow test method capable of detecting wheat, barley, and rye gluten residues at near equal levels down to 0.1 mg/swab and 1.0 ppm for food samples.

Purpose: To develop a rapid, sensitive, and reliable qualitative test method for detecting gluten residues obtained from environmental and food samples.

Methods: Polyclonal antibodies were raised against prolamin fractions derived from wheat, rye, and barley. The polysera from these individually immunized goats were immuno-purified and then blended. The antibodies were then used to develop a lateral flow test device (LFD) for detection of gluten from wheat, barley, and rye, configured with a test line, an overload line, and a procedural control. A test method was developed using a novel extraction buffer and a rapid extraction step (1 min at 95°C), and visualization after 15 min of running. Validation was performed to define test parameters and method concordance studies were performed using commercial ELISA kits based on R5 and Skerrit mAbs.

Results: The test method relies on a 1 min extraction at 95°C and an assay read at 15 min. The LOD for the device was determined as 0.1 ppm / swab and 1.0 ppm or food samples based on gliadin. The test was highly specific for prolamin residues derived from a panel of wheat, barley, and rye varieties, and did not cross-react with avenins isolated from R5(-) oats, sorghum, rice, soy, corn, millet or rice. The LFD was reproducibly more sensitive than the commercial ELISA kits, allowing for robust presumptive screening.

Significance: The MEI/IEH gluten test provides a rapid, highly specific, and extremely sensitive detection system for use in the food industry as part of routine HACCP monitoring and screening for gluten.

P1-77 Development and Characterization of Novel Monoclonal Antibodies Directed against Cereal Prolamins

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Introduction: Cereal grains contain a composite protein called gluten which consists of prolamins and glutelins. The prolamin fraction from wheat and related triticeae cereals exhibit immunopathogenic potential, and consumption of these grains is associated with symptoms of coeliac disease (CD). The current gold standard for detection of gluten residues used in the food industry relies on the R5 monoclonal, an antibody which over-estimates barley-derived gluten content and demonstrates cross-reactivity with common food commodities.

Purpose: In an effort to generate novel monoclonal antibodies with improved specificity profiles relative to the R5 system, we generated anti-prolamin monoclonals in mice. Herein, we describe the immuno-reactive profile of 8 candidate clones and compare activity against that of R5.

Methods: Prolamins from wheat, barley, and rye were purified using standard techniques and used to immunize female Balb/C mice. Spleens from seroconverted mice were fused with SP2/0-Ag14 cells and cultured using HAT techniques. Colonies were screened and selected based on reactivity towards gliadin, hordein, secalin, and avenin using indirect ELISA. IgG+ clones that retained high activity were raised and purified from ascites and further studied by ELISA and western blot analysis in conjunction with the R5 mAb.

Results: The hybridomas were functionally grouped as 1) Skerrit-like (3C1, 3G9, 10C10, and 7B5) by their preferential ability to detect gluten from wheat and rye over barley, 2) R5-like (28A4 and 25A5) based on their ability to pan detect gluten from wheat and rye over barley, and 3) unique (7E3, which equally detects gluten from barley and wheat, and 4F7, which preferentially detects barley over wheat). None of the clones demonstrated meaningful reactivity against corn, rice, and soy. Of the R5-like mAbs, 25A5 demonstrated essentially equal detection of gluten from wheat, rye, barley, and oats. In comparison, 28A4 demonstrated greatly diminished activity towards avenins.

Significance: Owing to limitations with the existing gluten-detection systems, we have generated a series of novel monoclonal antibodies directed against the prolamin fraction of triticeae cereals. Of the 8 clones tested, 28A4 and 25A5 demonstrated potential for use in future gluten detection systems, with divergent applications with respect to avenin detection.

P1-78 A Novel Universal Extraction Buffer for Use in Immunoassay-based Detection of Food Allergens and Gluten

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Introduction: Adverse immune responses to food allergens and gluten are relatively common and account for considerable morbidity and mortality. Consequently, the FDA has implemented food labeling requirements to inform consumers of potential health risks. To monitor for allergen and gluten contaminants, analytical food chemistry relies on the use of commercial, immune-based detection kits that isolate target analytes from food matrices utilizing extraction buffers. Considerable disparity exists in the performance of these extraction buffers, furthermore, no single extraction buffer has been advanced that can universally extract gluten as well as all food allergens.

Purpose: To formulate a universal extraction buffer that can efficiently and effectively recover key allergen and gluten residues from complex foods for downstream application in immunobased detection methods, including multiplexed detection assays.

Methods: A panel of complex foods containing milk, eggs, peanuts, tree nuts, shellfish, soy, wheat, and barley residues was compiled. Foods were extracted using our novel universal extraction buffer for 1 min at 95°C or using the commercial extraction buffers supplied by Neogen, ELISA Systems, and ELISA-TEK as per the respective test methods. Protein recovery rates were compared across systems using BCA protein assay, SDS-PAGE analysis, and ELISA.

Results: Extraction of egg, milk, shellfish, gluten, and soy residues using this novel buffer resulted in recoveries of 95-150% relative to the commercial buffers, depending on the food tested or the analytical method employed. In comparison, recovery of tree nut and peanut residues using this buffer was modestly reduced (~50%) relative to the commercial extraction buffers.

Significance: With increased compliance monitoring by FDA, we anticipate a growing need for multiplexed food allergen and gluten detection platforms. The development of a universal buffer for pan-extraction of food allergen residues and gluten is a critical step in the advancement of multiplexed detection platforms such as Bio-Plex® and lateral flow devices.

P1-79 Development and Validation of a Gluten ELISA Kit Using Polyclonal Antibodies Configured in Sandwich Format

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Introduction: Gluten has become a growing concern due to an apparent increase in the frequency of gluten intolerance. For this reason, the FDA has recently implemented regulations pertaining to gluten content in foods labeled "gluten-free." Existing gluten detection kits possess inherent limitations with respect to misestimation of barley residues (hordein) and/or cross reactivity with common food commodities. Accordingly, a rapid test kit capable of detecting gluten residues without cross-reacting concerns would greatly improve gluten HACCP for the food industry.

Purpose: To develop a rapid and facile sandwich-based ELISA kit for detection of gluten residues derived from wheat, barley, and rye which does not cross-react with common food commodities.

Methods: Polyclonal antibodies were raised against prolamin fractions from wheat, rye, and barley. The polysera from these individually immunized goats were immunopurified and then blended and developed into a sandwich-format immunoassay for the detection of gluten (wheat, barley, and rye) from complex food matrices. The final kit was validated and then compared with commercial Gluten ELISA kits based on Skerrit and R5 monoclonal antibodies.

Results: The MEI/IEH sandwich-based quantitative gluten ELISA kit consists of a 26 min test method (including extraction and assay operation). The kit detected spiked gluten residues (ROD 0.1 -100 ppm, $R^2 = 0.9686$) derived from wheat, barley, and rye at near equal stoichiometry. Validation analysis using a panel of prolamins derived from various cereals indicated that the kit is capable of broadly detecting gluten from relevant gluten sources without cross-reactivity with soy, sorghum, lupin, corn, or oats.

Significance: Gluten intolerance is a growing concern, as evidenced by recent efforts by the FDA and TTB to regulate gluten levels in foods labeled "gluten-free." The MEI/IEH sandwich-based gluten ELISA provides the food industry with an improved tool to rapidly and accurately detect contaminating gluten residues so as to ensure compliance with Federal regulations and provide safer foods for individuals with coeliac disease.

P1-80 The Development and Validation of a Novel Gluten ELISA Kit Based on Monoclonal Antibodies Configured in Competitive Format

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Introduction: In an effort to address growing public health concerns regarding gluten intolerance, the FDA and TTB have recently established regulatory threshold limits for gluten content in foods and alcoholic beverages labeled "gluten-free." Implementation and observance of these labeling laws require the use of antibody-based methodologies that can accurately quantify gluten levels in different food matrices. However, accurate quantification from fermented and/or hydrolyzed food products is problematic using conventional assay formats.

Purpose: To develop a rapid and accurate competitive ELISA kit for measurement of gluten residues derived from hydrolyzed and/or fermented wheat, barley, and rye food products.

Methods: Polyclonal antibodies were raised against prolamin fractions derived from wheat, rye, and barley. The polysera from these individually immunized goats were immunopurified on gluten-based affinity columns and then blended. The antibodies were then used to develop a competitive ELISA for the detection of gluten (wheat, barley, and rye) from complex food matrices including beer and sourdough. The finalized kit was validated using native and hydrolyzed gluten sources and then compared with a commercial competitive Gluten ELISA kit based on R5 monoclonal antibodies.

Results: We report that the MEI/IEH competitive ELISA kit could quantitatively detect gluten residues derived from wheat, barley, and rye within a range of quantification 1.5-100 ppm, $R^2 = 0.9801$, with near-stoichiometric detection for all three targets. The kit broadly detected distinct varieties of wheat, barley, and rye and did not cross-detect proteins derived from soy, sorghum, corn, lupin, or oats. The test method was performed in 36 min (extraction and assay operation). Method concordance with the R5-based kit demonstrated similar ability to detect hydrolyzed gluten residues in real food matrices, without concerns for over-estimation of hordein (barley).

Significance: This kit establishes a rapid and accurate method to detect hydrolyzed gluten residues in fermented foods and beverages in keeping with the regulations set forth recently by the FDA and TTB governing allowable gluten content in foods labeled "gluten-free." Implementation of this kit should particularly benefit the food and beverage industries by providing a more accurate measurement of hydrolyzed barley residues.

P1-81 A Novel Extraction Buffer for Use in Gluten Immunoassays

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Introduction: In an effort to address public health concerns regarding gluten, the FDA has recently established regulatory threshold limits for gluten content in foods labeled "gluten-free." Observance of this labeling law requires the use of immuno-based methodologies that can accurately quantify gluten levels in different food matrices. A key factor affecting the performance of these testing methods is the efficiency of the extraction buffer. For gluten, a class of proteins with unique solubility properties, extraction typically relies on the use of ethanol solutions (40-60%) to extract, thereby requiring additional dilution steps and presenting hazardous material concerns.

Purpose: To develop an effective gluten extraction buffer and extraction method for use in immunoassays that does not rely on the use of ethanol solution.

Methods: We developed an extraction buffer consisting of buffered 25% isopropanol and detergent that was directly compatible with downstream applications (including lateral flow assay and ELISA) without the need for dilution. In tandem, an extraction protocol was developed consisting of 1 min heat treatment at 95°C. Using a panel of complex food matrices, our buffer and test methods were directly compared with extraction/test methods from commercial ELISA kits based on Skerrit and R5 monoclonal antibodies.

Results: The MEI/IEH gluten extraction buffer performed comparably to the 40% ethanol (15 min. at 60°C) and 60% ethanol (~120 min. at room temperature) commercial extraction buffers when performed at 95°C for 1 min. In addition, as the MEI/IEH gluten extraction buffer is effectively buffered and formulated with lower solvent content, extracts can be directly applied to ELISA microwells and Lateral Flow Devices, allowing for more sensitive detection capabilities.

Significance: To obviate the need for 40-60% ethanol-based solutions, we have developed a novel extraction buffer based on 25% isopropanol content that yields efficient recovery results using a 1 min. extraction procedure without the need for additional dilution steps. With the increased demand for gluten residue testing following FDA regulations, we anticipate a need for a more rapid and streamlined extraction process.

P1-82 Validation of the First Marketed Multiplex Real-time PCR Rapid Test for *Salmonella* Enteritidis and Typhimurium Detection

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Introduction: BIOTECON Diagnostics developed and validated the foodproof® *Salmonella* Enteritidis & Typhimurium Detection LyoKit, a fast, sensitive and convenient assay for the detection and identification of the *Salmonella*serovars SE and ST in less than 24 hours. The pre-filled reaction mix is lyophilized, and the DNA sample preparation is added directly. It is the first multiplex real-time PCR rapid test on the market, which detects SE and ST simultaneously in one single test.

Purpose: Sensitivity, specificity, and robustness were determined in combination with the StarPrep One Kit for DNA extraction.

Methods: For exclusivity, more than 288 non-Enteritidis, non-Typhimurium *Salmonella* were successfully tested, including related serovars like e.g. Gallinarum. 50 non-*Salmonella* strains, including genetically related strains as well as strains commonly found in similar food environments, were also tested.

Results: SE was consistently detected at 10 GE/reaction or higher, while ST was detected at 5 GE/reaction or higher. Enrichments of 11 different food and PPS matrices like shell eggs, ground meat and chick dung were spiked with 10^2 - 10^3CFU/ml, both SE and ST were successfully detected. 60 and 58 strains of SE and of ST were successfully tested, respectively.

Significance: The new assay is a safe, easy-to-use and time-saving solution with proven sensitivity, specificity, and robustness.

P1-83 Identification of Leafy Green Recovered Bacteria with Antagonistic Activity against Enteric Bacterial Pathogens

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Developing Scientist Competition

Introduction: Interventions that prevent transmission of pathogens on produce surfaces by exploiting epiphytic pathogen-antagonizing native microbes could potentially assist in produce safety.

Purpose: The research objectives were to isolate and identify native bacteria on leafy greens exhibiting antagonistic activity against *Salmonella enterica* Typhimurium LT2 and *Escherichia coli* O157:H7 ATCC 700728.

Methods: Field samples of spinach and Endive lettuce (n = 30) were collected from two south Texas farms in June 2012. Of each sample, 25 g were taken and homogenized with 225 ml of 0.1% peptone water for 1 min. Aerobic mesophiles, lactic acid bacteria (LAB), fungi, enterococci, and coliforms were enumerated using appropriate media. From each sample, 4-12 isolated colonies from each medium were subjected to biochemical identification by Vitek 2 (bioMérieux N.A.). Pathogen-antagonizing isolates were identified using the Agar Spot method using de Man, Rogosa and Sharpe agar for LAB, or tryptic soy agar (TSA) for all other bacteria. Surface-spotted plates were incubated at 35°C for 24h; then overlaid with molten TSA containing 6.0 log CFU/ml *S.* Typhimurium or *E. coli* O157:H7. Plates were incubated at 35°C for 24h; inhibition halo diameters produced by isolates were measured by caliper, baseline corrected and averaged. Isolates producing a mean inhibition halo >1.0 mm were designated antagonistic.

Results: Populations of aerobic mesophiles, fungi, enterococci, LAB and coliforms were 6.7 ± 0.7, 5.3 ± 0.6, 3.4 ± 1.5, 4.7 ± 1.3 and 5.5 ± 0.9 log CFU/cm^2, respectively, for spinach; 6.1 ± 0.4, 5.0 ± 0.6, 2.7 ± 1.2, 4.6 ± 0.9 and 5.0 ± 0.5 log CFU/cm^2, respectively, for lettuce. Spinach-recovered isolates of *Staphylococcus intermedius* exhibited maximum antagonism against pathogens with inhibition halos ranging from 2.2 to 13.9 mm. Lettuce-recovered isolates of *Lactococcus garvieae* exhibited maximum antagonism with inhibition halos ranging from 1.1 to 17.8 mm.

Significance: These data suggest that native microorganisms present on the surfaces of leafy greens can inhibit pathogen growth and may be useful in ensuring produce safety.

P1-84 Microbial Assessment of Leafy Greens and Leafy Greens Contact Surfaces in Retail Foodservice Operations

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Introduction: With the increasing numbers of aging Americans, the emphasis on food safety "away from home" in retail foodservice operations that serve older adults has become paramount. Among the foods served, the safety of fresh produce items is of concern as leafy greens have been implicated in foodborne illness outbreaks and product recalls. Improper food handling in foodservice establishments is also major concern as it can lead to cross contamination and thereby illness.

Purpose: The objective of this study was to determine the food safety status of food contact surfaces and leafy greens handled at selected hospitals, restaurants, long term care facilities, and assisted living facilities in Kansas and Iowa.

Methods: Three leafy greens samples and three food contact surface samples were collected at four different types of foodservice operations in Iowa (n = 4) and Kansas (n = 4) as part of the food flow (receiving, handling and service). Microbiological analysis was contacted for presence/absence of *Listeria monocytogenes*, *Escherichia coli* O157, and *Staphylococcus aureus* using ELISA technology. Enumeration for aerobic plate counts (APC), coliforms, and fecal coliform (FC) counts using petrifilm was performed. Statistical analysis was performed using SAS program.

Results: All samples were negative for the pathogens tested. APC counts had significant difference for the type of sample ($P < 0.05$) with the leafy green samples having higher microbial counts than the surface samples (1.35-1.46 log CFU/g and 0.09-0.81 log CFU/cm^2, respectively). Coliform counts had significant difference between the types of operation, but at low levels (0-0.38 log CFU). FC counts were not significantly different for the type of sample and the type of operation (0-0.08 log CFU).

Significance: These results indicate that foodservices serving the elderly are procuring safe produce and are performing safe food handling practices but further behavioral studies may provide additional insight on the food safety status within these settings.

P1-85 Effects of Plant Growth Promoting Rhizobacteria on the Immune Response of Romaine Lettuce and Spinach toward Human Bacterial Pathoges

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Developing Scientist Competition

Introduction: *Bacillus subtilis* strain FB17 has been shown to trigger an induced systemic response (ISR) in *Arabidopsis thaliana* causing plant stomata to close and protecting the plant from infection by plant pathogens. FB17 treatment of plant roots has also been shown to close stomata as well as increase plant size and crop yield.

Purpose: The purpose of this study was to determine if FB17 was able to induce ISR in lettuce and spinach through stomatal closure during inoculation of human pathogens, *Listeria* spp, *Salmonella* spp., and plant pathogen *Pseudomonas syringae.*

Methods: Plants were grown in a controlled biochamber until 2 true leaves were present. Plant leaves were inoculated with sterile H_2O or bacterial pathogens only (1 x 10^8, *S.* Newport, *L. innocua*, or *P. syringae* DC3000); while, FB17 was inoculated onto the roots only, or on both leaves and roots. Two different leaves from each plant (n = 40) were collected at 1 and 3h post-inoculation (hpi) and were visually assessed using cryo-scanning electron microscopy (SEM) where stomatal aperture size was quantified using ImageJ software.

Results: Stomatal aperture size was significantly reduced compared to controls for all treatments indicating ISR was induced in lettuce and spinach ($P = < 0.0001$). ISR was not induced when lettuce was inoculated with FB17 on roots only at 1 and 3 hpi ($P = 0.23$), or with *Listeria* on lettuce only at 1 hpi ($P = 0.21$). However; FB17 triggered ISR at 3 hpi in the presence of human pathogens, thereby inducing stomatal closure in lettuce and spinach. Results were confirmed by root inoculation with heat-killed FB17, which did not induce stomatal closure. Significant differences were observed between plant species and pathogen interactions ($P < 0.0001$).

Significance: The results of this study indicate that plant growth promoting rhizobacteria *B. subtilis* FB17 may be able to prevent contamination by plant pathogens as well as internalization of human pathogens. This is the first study that assesses the effects of biocontrol agents and human pathogens on plants in terms of plant immune response through stomata.

P1-86 Persistence of *Eschericha coli* on Romaine Lettuce in Protected Agricultural Environments

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Introduction: The ability of *Escherichia coli* populations to survive in non-host habitats is greatly influenced by environmental conditions; including thermal variability, temperature fluctuations and nutrient availability. The survival of pathogens on lettuce grown in controlled atmospheric conditions may differ than survival on field grown lettuce.

Purpose: This study investigated the persistence of *E. coli* on lettuce plants grown in hoop houses.

Methods: Over two growing seasons, 385 Romain lettuce (var. Melody) plants grown in a hoop structure were inoculated with irrigation water contaminated with bovine manure containing *E. coli* between 0 and 10,000 CFU/ml. Coliform and *E. coli* present on lettuce leaves on days 0 (pre- and post-inoculation), 1, 3, 5, 7, 10 and 13 post-inoculation were determined.

Results: Immediately post-inoculation, concentration of *E. coli* on plants in all treatment groups increased to approximately 100 CFU/g. Concentrations of *E. coli* on days 1, 3, and 5 were inoculum-dose dependent and declined gradually to pre-inoculation concentrations (≤ 1 CFU/10 g) by one week post-inoculation; however, *E. coli* numbers subsequently increased to 3 CFU/g on plants inoculated with the highest concentrations of *E. coli.*

Significance: Low microbial quality irrigation water may introduce microorganisms to the surface of lettuce. Although counts tended to decline over time, even under the controlled conditions of hoop house productions, *E. coli* populations may fluctuate dramatically as a result of factors that are yet to be determined.

P1-87 Fate of *Salmonella enterica* in a Mixed Ingredient Salad Containing Lettuce, Cooked Chicken and Cheese

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Poster

Introduction: The food service and retail sectors offer consumers an expanding variety of mixed ingredient salads that are increasingly assembled at the point of sale from fresh-cut vegetables and prepared ingredients obtained from external suppliers. Additional ingredients may include fruits, nuts, cereals, eggs, dairy products, cooked seafood and cooked or processed meats, among others. Little is known about the fate of enteric bacterial pathogens in mixed ingredient salads.

Purpose: To examine the fate of *Salmonella enterica* in a mixed ingredient salad containing fresh-cut lettuce, cheddar cheese and cooked chicken.

Methods: Eight mixed salads (500 g) in polyethylene trays containing fresh-cut Romaine lettuce overlaid with cooked chicken and cheddar cheese were obtained from a local retailer on two occasions. The ingredients were separated, one third of the lettuce from each salad was discarded and replaced with an equivalent weight of lettuce washed in chlorinated (70 ppm Cl) water after inoculation with a five strain cocktail of *S. enterica.* The cooked chicken and cheese were returned to one half of the salads and *S. enterica* populations were estimated on XLD agar in each salad immediately and after 3 days of storage at 6°C or 14°C.

Results: *S. enterica* populations declined slightly in salads stored at 6°C without added ingredients (2.19 ± 0.11 to 1.52 ± 0.51 log CFU/g) and with cooked chicken and cheese (1.76 ± 014 to 1.08 ± 0.15 log CFU/g). In contrast, *S. enterica* grew in salads stored at 14°C and the population increase was significantly ($P < 0.05$) higher in salads with added ingredients (4.16 ± 0.45 log CFU/g) than lettuce alone (1.75 ± 0.58 log CFU/g).

Significance: The finding that growth of *S. enterica* was accelerated in a mixed ingredient salad highlighted the need to ensure these products are formulated with ingredients free of enteric bacterial pathogens and emphasized the critical importance of temperature control during their storage and distribution.

P1-88 Survival of Norovirus Surrogates on Lettuce: A Comparison among Feline Calicivirus, Murine Norovirus, Porcine Sapovirus and Tulane Virus

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Introduction: Human norovirus (HuNoV) is the leading cause of foodborne illnesses. The virus cannot be cultured; therefore, its survival on leafy greens cannot be assessed. Several HuNoV cultivable surrogates have been proposed, including: Feline calicivirus (FCV), murine norovirus (MNV), porcine sapovirus (SaV) and Tulane virus (TV).

Purpose: To compare the survival of FCV, MNV, SaV and TV on lettuce leaves post-harvesting.

Methods: Viruses were propagated in cell culture and harvested in the media (untreated). To accurately compare these viruses, we removed the cell culture media by ultrafiltration using Amicon® centrifugal filter devices (100K) followed by re-suspension in original volume with sterile water. The survival of untreated as well as ultrafiltered viruses was compared: (i) in suspension at room temperature (RT) for 28 days; and (ii) following spot inoculation onto lettuce leaves at RT (for 3 days) and 4°C (for 14 days). Virus infectivity titers were determined by end-point dilution.

Results: At RT, untreated FCV, SaV and TV lost infectivity by days 7, 14 and 21; respectively, while MNV remained infectious through day 28. In contrast, ultrafiltered viruses remained infectious through at least day 28 with 0.05, 1.2, 1.9 and 2.03 log reduction in infectious titers for MNV, TV, FCV and SaV, respectively When the untreated viruses were inoculated onto lettuce leaves for 3 days at RT, MNV was significantly more stable than SaV and FCV (1.1 versus 2.5 and 2.7 log reduction in infectious titer, respectively). In contrast, ultrafiltered viruses showed no significant difference in survival by day 3. Inoculation of leaves with the untreated viruses at 4°C for 14 days showed that MNV was significantly more stable than SaV and FCV; however, ultrafiltered MNV showed similar survival to SaV and FCV.

Significance: Our results suggest that after reconstitution in water, all surrogate viruses survived similarly on lettuce leaves.

P1-89 Effect of Different Mulches Used for Growing Organic Lettuce on Soil and Epiphytically-associated Fecal Indicator Bacteria

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Developing Scientist Competition

Introduction: A variety of cropping methods are used by small farmers to grow lettuce in the Mid-Atlantic region of the U.S., but their impact on enteric human pathogens remains largely unexplored. The use of mulches to retain moisture and control weeds is a common practice, but the influence of various mulches on enteric pathogen survival and dispersal is unknown.

Purpose: The goal of this study was to assess the impact of different mulching methods on the survival of soil and epiphytically-associated fecal indicator bacteria on organically grown lettuce during different growing seasons (spring and fall).

Methods: Lettuce was grown under organic practices using various mulches (polyethylene plastic, corn-based biodegradable plastic, paper mulch) and bare ground in a randomized complete block design. At 8 weeks post-transplanting, lettuce was inoculated with manure containing known levels of generic *Escherichia coli* and *Enterococcus* spp. Leaves and soil samples were collected at intervals over a 2-week period, and quantitatively analyzed for *E. coli* and fecal coliforms on Tryptone Bile X-Glucuronide (TBX) agar and *Enterococcus* spp. on Enterococcosel agar.

Results: Bacterial concentrations decreased under all treatments, with more rapid declines in the fall than in spring (slope = -0.75 in fall and - 0.38 in spring). Within each season, no significant differences in the persistence of *E. coli*, fecal coliforms and *Enterococcus* was observed among the three types of mulches and bare ground in the rhizosphere and phyllosphere. However, a significant difference was observed between spring and fall seasons.

Significance: This study demonstrates that mulches used in lettuce production are not likely to impact the fate of enteric pathogens in soil or on lettuce. However, the data suggest that the time between exposure to a source of enteric bacteria and harvesting of the crop is seasonally dependent and has implications for determining best harvest times.

P1-90 The Impact of Solids on the Efficacy of Chlorine in Preventing *Salmonella* Cross-contamination during Washing of Fresh-cut Lettuce

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Developing Scientist Competition

Introduction: Fresh-cut vegetables are commonly washed in chlorinated water to remove dirt, plant debris, and other agricultural chemicals. As the wash water is often recycled during commercial processing, its quality can change due to increases in solids and other extraneous material. How this change can affect the efficacy of chlorine in preventing microbial cross-contamination needs to be determined.

Purpose: Determine how the presence of solids may affect chlorine inactivation of *Salmonella* and the potential for pathogen cross-contamination during washing of fresh-cut lettuce.

Methods: Small-scale experiments were conducted to determine the impact of different types of soil in affecting the efficacy of chlorine in disinfecting *Salmonella*. 100 ml sterile DI water containing 2 – 20 g of play sand or 1 – 5 g of sandy soil was inoculated with 6 log CFU/ml *Salmonella* and treated with different levels of free chlorine (0.5 - 20 ppm) for 15 sec. Lettuce washing experiments were also performed to determine whether the presence of solids affected the efficacy of chlorine in preventing *Salmonella* cross-contamination. Eight g of cut romaine lettuce inoculated with 7 log CFU/g of *Salmonella* was added to 40 l of sterile tap water together with 800 g of uninoculated cut lettuce and washed for 2 min. Washing trials were performed with different levels of chlorine (0 and 10 ppm) and play sand (0 and 4 kg).

Results: In water containing 8 ppm of chlorine, the level of *Salmonella* changed from not detectable in the presence of < 5 g of play sand to 0.3 or 1.4 log CFU/ml in the presence of 10 or 20 g of play sand, respectively. At 10 ppm of chlorine, *Salmonella* level changed from not detectable to 1.0 log CFU/ml when the level of sandy soil increased from 0 to 5 g. In 40 l washing trials without chlorine treatment, *Salmonella* transfer occurred and resulted in the contamination of wash water and uninoculated lettuce at levels of 2.9 ± 0.1 log CFU/ml and 2.9 ± 0.3 log CFU/g, respectively. With 10 ppm of chlorine, no *Salmonella* was detected; but when 4 kg play sand was added, although no *Salmonella* was detected in the lettuce samples, it was found in the wash water after enrichment.

Significance: The presence of solids provides protection for *Salmonella* thus avoiding inactivation by chlorine, and may affect the sanitizer's ability to prevent cross-contamination.

P1-91 Effectiveness of a Batch Ozonated Retail Wash System for Iceberg Lettuce

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Introduction: Retail companies are exploring new ways to improve their in-store safety performance through the application of preventative controls when processing fresh produce.

Purpose: In response to this interest, ozone was evaluated during simulated iceberg lettuce processing for its effect on surface-adhered and planktonic bacteria, including naturally-occurring bacteria on head lettuce and inoculated human pathogens.

Methods: 22 heads of cored iceberg lettuce were submerged in 75 l of tap water and covered with 5400 g of ice, after which ozone was introduced with a recirculating injection system. After a 20 min rinse and hydration interval, 2 heads of lettuce were removed every 2 min, with inner and outer leaves immediately neutralized for microbial analysis. Dissolved ozone was measured every 10 min (Hach AccuVac #73), and lettuce (20, 26, 32, and 38 min of washing) and water samples (collected every 10 min) were quantified for total heterotrophs and coliforms. The same procedures were followed using lettuce inoculated with ~4.5 log CFU/g of *E. coli* O157:H7, *L. innocua*, and *Salmonella* Typhimurium. In both experiments, wash water parameters were measured every 10 min.

Results: Wash water containing ozone resulted in significantly lower ($P < 0.05$) populations of total heterotrophs and coliforms after 30 min (0.2 log CFU/ml and 0.6 log MPN/100 ml) when compared to the water control (2.5 log CFU/ml and 2.9 log MPN/100 ml), although pathogen populations did not differ ($P > 0.05$). Ozone doses applied did not have a significant effect on surface-adhered bacteria. Of the parameters studied, ORP was the most affected by continual ozone injection, with significantly higher ($P < 0.05$) values 10 through 40 min after ozone generation began.

Significance: The use of ozone can help alleviate bacterial build-up in the water and potentially aid in preventing cross-contamination, fulfilling goals for preventative controls in small-scale food preparation.

P1-92 Withdrawn

P1-93 Effectiveness of Listex™ (P100) against *Listeria monocytogenes* on Spinach Leaves

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Introduction: The percentage of outbreaks related to leafy vegetables occurring from 2006-2008 has increased significantly compared to the two year period from 1998-1999. This increase in outbreaks correlates to the growing amount of salad consumed per year, now totaling over 40 billion servings each year in the United States, with almost 3 billion of that being fresh spinach. Current washing methods for spinach do not adequately inhibit microbial growth and research must be done on novel approaches for creating a safe product for consumers.

Purpose: The purpose of this study was to determine the efficacy of Listex (P100) against *Listeria monocytogenes* on spinach leaves when used as a spraying and dipping treatment.

Methods: Triple-wash dipping treatments (chlorine:chlorine:chlorine, chlorine:tap-water:tap-water or chlorine-tap-water-phage), and a spraying treatment with or without a chlorine pre-wash, were tested against *L. monocytogenes* on spinach. The phage was used at a concentration of 10^8 PFU/ml for the dipping treatments and 10^8, 10^9 or 10^{10}PFU/ml for the spray treatments. There were 30 and 60 s phage contact times for the spray treatment and a 30 s contact time for the dipping treatment.

Results: A chlorine:tap-water:phage dip was of varying effectiveness, resulting in a 1.10 to greater than 3.29-log reduction of *L. monocytogenes*. Replacing the tap-water wash with a phage wash in the triple-wash system decreased *Listeria*, on average, by only an additional 0.55 log CFU/g. Spraying spinach resulted in an approximate 1 log reduction in *Listeria* after 24h when using the bacteriophage at a concentration of 10^9PFU/ml.

Significance: These data suggest that the cost:benefit of adding these treatments to a processing line would need to be considered with respect to the minimal effectiveness of the phage against *L. monocytogenes* on spinach leaves.

P1-94 Surface Texture Analysis of Fresh Produce using Optical Interferometry

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Introduction: The methods to reduce pathogens on fresh produce are of limited effectiveness, and development of new methods are challenging given the variable surface topography of fruits and vegetables. Evaluating the surface characteristics of produce can aid in understanding contamination and potentially lead to the development of novel strategies for decontamination. While previous microscopy methods utilized to

evaluate produce surface characteristics involve extensive sample preparation and are destructive, optical interferometry provides an alternative method that requires no sample preparation and is nondestructive.

Purpose: The purpose of this study was to use an optical interferometer to compare the surface characteristics of Romaine lettuce, spinach, cilantro and tomatoes.

Methods: The objective lens on the optical interferometer was 20X magnification and the scan size was 0.32 x 0.24 mm. Leafy vegetable measurements were taken of the top and bottom of leaves, while tomatoes were only tested at one location. Five measurements per test condition were collected and the 2D and 3D surface parameter values in the roughness scale were compared using ANOVA.

Results: The 2D average roughness (R_a) values were 7.25 ± 1.67, 6.98 ± 1.73, 3.51 ± 0.91, 3.14 ± 0.99, 8.80 ± 1.07, 9.19 ± 0.46 and 1.43 ± 1.45 µm for the top of Romaine lettuce, bottom of Romaine lettuce, top of cilantro, bottom of cilantro, top of spinach, bottom of spinach and tomatoes, respectively. There was a significant difference ($P > 0.05$) in R_a between the top of all the products except for Romaine lettuce and spinach. Tomato had the smallest average peak-to-valley height (R_z) followed by bottom of cilantro, top of cilantro, top of Romaine lettuce, bottom of Romaine lettuce, bottom of spinach and top of spinach.

Significance: These data suggest that optical interferometry is a quick and effective means to evaluate the surface characteristics of fresh produce.

P1-95 Sensitivity of *Pseudomonas fluorescens* to Gamma Irradiation Following Surface Inoculations on Romaine Lettuce and Baby Spinach

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Introduction: Foodborne pathogens are major sources of contamination of fresh produce and other minimally processed food types. Irradiation of fresh fruits and vegetables is a post-harvest intervention measure often used frequently to inactivate pathogenic foodborne microbes.

Purpose: The purpose of this study was to assess the effects of gamma irradiation on D_{10} values of *P. fluorescens* surface inoculated on romaine lettuce and baby spinach, and the sensitivity of the bacteria (D_{10} values) suspended in buffer peptone water.

Methods: Gamma irradiation was applied on romaine lettuce and spinach following surface inoculations with *P. fluorescens* (Pf) strains (2-79, Q8R1, and Q287) at doses of 0 (control), 0.25, 0.5, 0.75 and 1.0 kGy and the leafy greens were subsequently stored for 24 hrs at 5°C. The effects of gamma irradiation were also determined on bacteria cells suspended in buffered peptone water (BPW) in tubes. The Pf strains are non-plant pathogenic, non-pectolytic, and saprophytic in nature.

Results: The initial *Pseudomonas* populations ranged from 9.08 ± 0.21 to 9.69 ± 0.16 log CFU/ml of BPW in the non-irradiated control. The bacterial counts on the non-irradiated spinach and romaine lettuce ranged from 8.14 ± 0.34 to 8.95 ± 0.06 and from 8.32 ± 0.05 to 8.63 ± 0.25 log CFU/g, respectively. The radiation D_{10} values of *P. fluorescens* suspended in BPW ranged from 0.09 ± 0.00 to 0.12 ± 0.01 kGy when enumerated on *Pseudomonas* Agar F and Trypticase Soy Agar. However, on spinach and romaine lettuce, the D_{10} values of *P. fluorescens* were significantly ($P < 0.05$) lower and ranged from 0.04 ± 0.01 to 0.05 ± 0.02 and from 0.05 ± 0.03 to 0.06 ± 0.03 kGy, respectively.

Significance: There was high sensitivity of *P. fluorescens* (the biocontrol microbe) to irradiation when inoculated on surfaces of romaine lettuce and spinach. In future, combinations of low dose irradiation and biocontrol treatments will be investigated to enhance the potential synergistic inactivation of foodborne pathogens.

P1-96 Seed Disinfection in Sprout Production: Survey of Methods Applied, Basis for Selection and Efficacy in Controlling Human Pathogens

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Introduction: Sprouted seed such as alfalfa have been implicated in high profile foodborne illness outbreaks and as such are considered a high risk food despite the perceived nutritional benefits. In addition to applying GAP during production, pathogen screening and traceability, seed disinfection is considered a key intervention to enhance microbiological safety of sprouts. Currently seed disinfection is voluntary in sprout production although proposals under FSMA would make a verified seed disinfection method mandatory in the industry.

Purpose: To undertake a survey on what seed disinfection methods are currently applied in industry and understand the reasons for selection. A second part of the study tested the efficacy of the identified seed disinfection methods to control *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes*inoculated on alfalfa seeds.

Methods: A thirteen-question survey was sent to sprout growers in North America, Europe and Australia to identify seed disinfection applied, in addition factors influencing selection. Laboratory based trials assessed the efficacy of identified seed disinfection methods to inactivate human pathogens inoculated onto alfalfa seed. Assessment of the different seed disinfection methods was assessed on the basis of effect on seed germination, initial reduction of pathogens and potential for post-treatment recovery during the sprouting process.

Results: From the survey results it was found that 90% of respondents applied seed disinfection with hypochlorite being the most used although hot water, peroxyacetic acid, hydrogen peroxide, electrolyzed water and chlorine dioxide were also applied. The main motivation for selecting a specific seed disinfection method was based on government recommendations. From laboratory trails none of the treatments, apart from stabilized sodium chlorite, could ensure elimination of pathogens without post-treatment recovery or significant effect on germination rates.

Significance: Methods for seed disinfection should be evaluated using standard protocols taking germination rates and potential pathogen post-treatment recovery into account. Government recommendations have a strong influencing the method sprout producers used for seed disinfection.

P1-97 Growth of *Escherichia coli* O157: H7 and O104: H4 during Sprouting and Microgreen Production from Inoculated Radish Seeds

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Introduction: Sprouts and microgreens are popular tender produce items, typically grown in indoor facilities which allow a higher degree of control compared to open field production. While sprout have frequently been implicated in foodborne illness outbreaks, there is a lack of data pertaining to the microbiological safety of microgreens.

Purpose: The primary objective of this work was to compare the survival and proliferation of *Escherichia coli* O157: H7 and O104: H4 on radish sprouts and microgreens cultured in a BSL-2 growth chamber simulating commercial sprout and microgreen production conditions.

Methods: In this study, sprouts and microgreens were produced from radish seeds inoculated with *Escherichia coli* O157: H7 and O104: H4 and *E. coli* populations on the harvested products compared using spiral plating and most probable number (MPN) methods with four replicates.

Results: Both *E. coli* O157:H7 and O104:H4 proliferated rapidly during sprouting, reaching levels of 5.8 to 8.1 log CFU/g and 5.2 to 7.3 log CFU/g, respectively, depending on the initial inoculation levels of the seeds. In comparison, *E. coli* O157:H7 and O104:H4 populations on harvested microgreens ranged from 0.8 to 4.5 log CFU/g and from 0.6 to 4.0 log CFU/g, respectively. Although proliferation of *E. coli* O157:H7 and O104:H4 could occur during both sprouting and microgreen growth, harvested microgreens carried significantly less ($P < 0.001$) *E. coli* than sprouts produced from radish seeds contaminated at corresponding levels.

Significance: This study provided the scientific data comparing and contrasting the food safety risks of sprouts and microgreens. It underlined the importance of maintaining the microbiological quality of seeds, especially those used for sprouting.

P1-98 Comparison of the Efficacy of Three Organic Sanitizers with 20,000 ppm Calcium Hypochlorite for Inactivation of *Salmonella* on Artificially Contaminated Alfalfa Seeds

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Developing Scientist Competition

Introduction: Although treatment of seeds with 20,000 ppm calcium hypochlorite, $Ca(OCl)_2$, is considered the gold standard, the high level of chlorine is potentially hazardous. Sprout growers are in need of alternative sanitizers. Many organic sanitizers have been approved for disinfection of produce; whether they perform equally well in disinfection of seeds for sprouting needs to be determined.

Purpose: Evaluate the efficacy of three organic sanitizers, acidified sodium chlorite (ASC, also known as Sanova), stabilized oxychloro-based sanitizer (SOC, also known as Germin-8-or), and the organic CHICO wash, in comparison with 20,000 ppm $Ca(OCl)_2$ for disinfection of alfalfa seeds artificially inoculated with *Salmonella*. Small-scale experiments were conducted to determine the best treatment conditions. Large-scale studies were performed to evaluate the commercial applicability of these sanitizers.

Methods: Ten g of seeds inoculated with 3-4 log CFU/g of *S.* Typhimurium expressing green fluorescent protein was treated with different concentrations of ASC, SOC, and the CHICO wash for different periods of time. For large-scale runs, 1 kg of seeds spiked with 1 % (w/w) of inoculated seeds was treated with each sanitizer under optimal treatment conditions. As a comparison, seeds were also treated with 20,000 ppm $Ca(OCl)_2$ for 15 min. After treatments, seeds were rinsed twice with sterile tap water and analyzed for *Salmonella* by plate counts or the 3-tube MPN method.

Results: The efficacy of ASC and SOC increased with increasing concentrations (up to 1500 ppm) and treatment time (up to 24h); however, the germination rates for seeds treated with > 1200 ppm of either sanitizer were < 80%. Treatment with 800 ppm of ASC or SOC for 3h reduced the *Salmonella* level, respectively, by 2.3 logs or > 2 logs to an undetectable level, compared with the ~1 log decrease observed in seeds treated with 20,000 pm $Ca(OCl)_2$. Treatment with 15% (v/v) CHICO wash for 10 min lowered the germination rate to 73% but only reduced the *Salmonella* count by 0.9 log. For the large-scale runs, the *Salmonella* level in seeds treated with 800 ppm SOC for 3h decreased by > 1.9 log to an undetectable level (< -2.52 log MPN/g) compared with a 1.5 log reduction in seeds treated with 20,000 ppm $Ca(OCl)_2$.

Significance: The stabilized oxychloro-based sanitizer may provide an alternative seed treatment method for organic sprout growers.

P1-99 Survival and Growth of *Salmonella* during Sprouting of Alfalfa Seeds as Affected by Seed Treatment with 20,000 ppm Calcium Hypochlorite and Sprouting Conditions

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Developing Scientist Competition

Introduction: It has been reported that treatment with 20,000 ppm of calcium hypochlorite, $Ca(OCl)_2$, may not completely eliminate pathogens on seeds; however, the conditions that determine the extent of pathogen re-growth during sprouting have not been elucidated.

Purpose: Examine whether the contamination level on seeds affects the extent of *Salmonella* proliferation during sprouting of seeds treated with 20,000 ppm $Ca(OCl)_2$, and determine how pathogen proliferation may be minimized by adjusting sprouting conditions (irrigation frequency, sprouting temperature, and irrigation with chlorinated water).

Methods: 1200 g of alfalfa seeds spiked with 1 % of inoculated seeds that contain ~ 1 - 5 log CFU/g of *Salmonella* were treated with 20,000 ppm $Ca(OCl)_2$ for 15 min. The treated seeds were germinated either in a glass jar or in an automatic sprouter (EasyGreen®) for 3-5 days at room temperature (~22°C) or 30°C, and irrigated with tap water or water containing 100 ppm of sodium hypochlorite at different frequencies (once every 2, 4 or 24 hours). Sprouts samples were taken daily and analyzed for *Salmonella*, either by plating on XLD or by the three-tube MPN method described in FDA BAM.

Results: For seeds with a low contamination level (~ -1 log CFU/g), treatment with 20,000 ppm $Ca(OCl)_2$ lowered the level of *Salmonella* by > 2 log units to an undetectable level. No *Salmonella* growth was observed during 5 days of sprouting, regardless of whether the sprouts were irrigated with tap water or chlorinated water. For seeds with a high contamination level (~1 log CFU/g), the *Salmonella* level was also reduced by ~2 logs after seed treatment; however, the level in sprouts increased significantly under all sprouting conditions. Germination in glass jars at 30°C resulted in the greatest *Salmonella* growth (by ~5 log CFU/g). For sprouts grown at room temperature, irrigation with chlorinated water did not prevent *Salmonella* proliferation.

Significance: Seed treatments with 20,000 ppm $Ca(OCl)_2$ have the potential to reduce pathogen load on seeds to levels low enough such that re-growth does not occur. Adjusting sprouting conditions especially temperature could reduce pathogen proliferation during sprouting.

P1-100 Inactivation of Human Norovirus and Its Surrogates on Alfalfa Seeds by Aqueous Ozone

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Developing Scientist Competition

Introduction: Alfalfa sprouts have been associated with numerous foodborne outbreaks, where seed contamination was identified as the source. There is a gap in knowledge regarding viral contamination of seeds since human norovirus is a leading cause of produce-associated outbreaks. Many studies have been conducted investigating the effectiveness of aqueous ozone on bacterial-contaminated seeds, yet little is known about viruses.

Purpose: This study assessed aqueous ozone for the disinfection of alfalfa seeds contaminated with human norovirus (huNoV) and huNoV surrogates. The inactivation of viruses without food matrix was also investigated.

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Methods: Alfalfa seeds were inoculated with huNoV GII, Tulane virus (TV) and murine norovirus (MNV); viruses without food matrix/inoculated seeds were soaked in deionized water containing 6.25 ppm aqueous ozone with agitation at 22°C for 30s, 1, 5, 15, or 30 min. Sodium thiosulfate (5%) was added post-treatment to quench residual ozone; samples were collected, and seed viruses eluted with Hank's Balanced Salt Solution. Viruses were quantified by real-time RT-PCR and plaque assay; inoculated seeds soaked in agitated water without ozone were included as controls.

Results: Aqueous ozone resulted in inactivation of MNV and TV infectivity from 1.66 ± 1.11 to 5.60 ± 1.11 log PFU/g seeds; for all treatment times significantly higher reductions were observed for MNV ($P < 0.05$). Viral genomes were relatively resistant with reduction of 1.03 ± 0.54 to 3.00 ± 0.14 log genomic copies/g seeds; reduction of TV was similar to that of huNoV, whereas the MNV had significantly greater reductions in genomic copies ($P < 0.05$). Similar trends were observed in ozone-treated viruses without food matrix, with significantly higher levels of inactivation ($P < 0.05$), especially with reduced levels of infectivity for MNV and TV.

Significance: Significant inactivation by aqueous ozone indicates that ozone can be an alternative treatment for seeds. The behavior of TV similar to huNoV makes it a promising surrogate.

P1-101 Attachment and Biofilm Formation by Selected Strains of *Salmonella enterica* and *Escherichia coli* of Fresh Produce Origin

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Introduction: *Salmonella enterica* and *Escherichia coli* have both been linked to fresh produce-related, especially sprout-related, outbreaks of infections. Cells of the two pathogens are capable of adhering to and forming biofilms on their contact surfaces including food surfaces.

Purpose: In this study, we compared the biofilm-forming abilities of selected *S. enterica* and *E. coli* strains of fresh produce origin on polystyrene surface and their adherence to alfalfa and bean sprouts.

Methods: Overnight cultures (2 ml) of seven *S. enterica* and four *E. coli* strains in six different broths were placed into polystyrene tissue culture plates for biofilm development for 2 to 7 days at 28°C. The biofilms formed were quantified using the crystal violet binding assay. In a separate experiment, alfalfa and bean sprouts (5 g) were exposed to 25 ml of overnight cultures of *S. enterica* or *E. coli* at 22°C for 2h with shaking at 40 rpm. Contaminated sprouts were rinsed twice with sterile water and homogenized with a stomacher at normal speed for 30 sec. Bacteria attached to sprouts were enumerated.

Results: Biofilm mass on polystyrene surface increased with incubation time ($P < 0.05$) with some exceptions. Among the six microbiological media used, LB no salt broth better supported biofilm development ($P < 0.05$). Two *E. coli* strains formed more biofilms than the *Salmonella* and other two *E. coli* strains ($P < 0.05$). In the attachment study, more *Salmonella* (5.66 log CFU/g) than *E. coli* (3.46 log CFU/g) cells attached to both sprouts ($P < 0.05$). Cells of both pathogens attached in higher numbers to bean sprouts compared to alfalfa sprouts ($P < 0.05$).

Significance: Pathogens with relatively poorer initial adherence can sometimes develop more biofilms than the stronger colonizers. Since native biofilms are abundant on food surface, the potential public health threats posed by the poorer colonizers should be considered.

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P1-102 Effectiveness of Chlorine Dioxide Gas Treatment in Inactivating *Salmonella enterica* on Mungbean Sprouts

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Introduction: Fresh-sprouted beans and grains are considered a good source of nutrients. However, they have been associated with foodborne outbreaks. Sprouts provide a good matrix for microbial localization and growth due to optimal conditions of temperature and humidity while sprouting. Also, the lack of a kill step post-sprouting is a major safety concern.

Purpose: The objective of this work was to evaluate the effectiveness of chlorine dioxide (ClO_2) gas treatment to reduce surface *Salmonella* on mungbean sprouts.

Methods: Mungbean sprouts (4 days old) were artificially inoculated with a cocktail of *Salmonella enterica* (serovars Infantis, Meunchen, and Newport) sprout related isolates. The effectiveness of inactivating *Salmonella* cells on the inoculated sprouts using gaseous ClO_2 (0.5 mg/l air) with or without tumbling (mechanical mixing) treatment was compared to an aqueous chlorine (200 ppm) wash treatment at room temperature.

Results: Tumbling the inoculated sprouts during the ClO_2 gas application for 15, 30 and 60 min reduced *Salmonella* populations by 3.0, 4.0 and 5.5 log CFU/gram, respectively, as compared to 3.0, 3.0, and 4.0 log CFU/gm reductions obtained without tumbling, respectively. A 2.0-log CFU/gm reduction in *Salmonella* was achieved with aqueous chlorine wash. The difference in microbial reduction between ClO_2 gas vs. aqueous chlorine wash points to the important role of surface topography, pore structure, bacterial attachment to inaccessible sites, and/or biofilm formation on sprouts.

Significance: The data presented here suggested that ClO_2 gas was capable of penetrating and inactivating cells which are attached to inaccessible sites and/or are within biofilms on the sprout surface as compared to an aqueous chlorine wash. Consequently, scanning electron microscopy imaging indicated that ClO_2 gas indeed was capable of penetrating and inactivating cells attached to inaccessible sites on the sprout surfaces.

P1-103 Inactivation of *Salmonella enterica* on Artificially Inoculated Mature Green Tomatoes: Efficacy and Cost Modeling of Gaseous Chlorine Dioxide

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Introduction: Outbreaks of foodborne illnesses have been associated with the consumption of tomatoes contaminated with *Salmonella*. Commercial washing processes for tomatoes are limited in their ability to inactivate and/or remove this human pathogen.

Purpose: Our objectives were to demonstrate the efficacy of chlorine dioxide (ClO_2) gas for inactivation of *Salmonella* on green tomatoes and its effect on maturation quality, and to develop analysis for this process.

Methods: Mature green tomatoes were dip inoculated with *Salmonella* Montevideo and were stored at 13°C for 24h prior to processing. Gaseous ClO_2 treatments of inoculated tomatoes were conducted at 0.2, 0.4, and 0.8 mg/l air for 2, 4, and 8h. Treated and non-treated tomatoes were then stored in modified atmosphere (97% Nitrogen, 3% Oxygen) at 13°C and 90% relative humidity for 5 days, and then were stored for an additional 7 days in a standard atmosphere at 21°C and 75% relative humidity to allow for ripening.

Results: Gaseous ClO_2 treatments significantly reduced *Salmonella* populations in excess of 3.0 log CFU/g tomato. Color and texture profiles of ripened treated tomatoes were not significantly different from the ripened control samples. The cost estimates under the laboratory conditions used indicated that this treatment might add $0.17 per kg tomato.

Significance: These results indicate that ClO_2 gas phase treatments could enhance the microbiological safety of tomatoes and extended the shelf life without affecting the ripening process. Although, an increase of $0.17 in cost per kg might be considered to be prohibitive at this point, the decrease in economic losses due to extending the shelf life, and the adoption of this technology under commercial production conditions would reduce these associated costs.

P1-104 Prevention of the Internalization of *Salmonella* spp. in Growing Mung Bean Sprouts with the Vegetable-associated *Bacillus subtilis* Strain LCA1

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Introduction: Consumption of seed sprouts has been associated with multiple foodborne disease outbreaks. Current disinfection of seeds, prior to sprouting, with chemicals such as calcium hypochlorite, is not fully effective because pathogens such as *Salmonella* spp. can internalize in plant tissues and, thus, be protected from surface sanitization.

Purpose: This study is to explore the potential of using vegetable-associated *Bacillus subtilis* strains as bio-agents to prevent the internalization of *Salmonella* spp. in growing mung bean sprouts.

Methods: With the deferred agar spot method, *Bacillus subtilis* strains isolated from lettuce and mung bean seeds were screened for antimicrobial activities against common foodborne pathogens, including strains of *Salmonella spp.,* Shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes.*

To evaluate the efficacy of the *B. subtilis* isolate LCA 1 for inhibiting the internalization of *Salmonella* spp. in growing mung bean sprouts, mung bean seeds were incubated with the LCA 1 culture suspension and then inoculated with cells of *S. enterica* ser. Typhimurium ATCC14028 before sprouting. The spouts were withdrawn at predefined intervals to enumerate the *Salmonella* Typhimurium by plating sprout homogenates on selective agar plates after surface sanitization.

Results: The *B. subtilis* isolate LCA1 showed broad inhibition spectrum against *Salmonella* spp., *E. coli*, and *L. monocytogenes* with the radii of the inhibition zones ranging from 2 to 13 mm on the agar plates. The *B. subtilis* isolate LCA1 significantly reduced the internalized *Salmonella* load in the growing spouts. The maximal reduction occurred at day 2 of sprouting, where the LCA1-treatment group appeared to have 60 times fewer internalized *Salmonella* load, compared with the control group.

Significance: The results suggest that the vegetable-associated *Bacillus subtilis* LCA 1 may be used as a bio-agent to control internalization of foodborne pathogens in growing mung bean sprouts.

P1-105 Comparison of the Efficacy of Various Sanitizers and Hot Water Treatment in Inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and Natural Microflora on Mung Bean Sprouts

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Introduction: The microbiological safety of sprouts has been a concern in recent years following several reports of foodborne outbreaks associated with consumption of sprouts. The frequent occurrence of outbreaks suggests the need for effective decontamination of sprouts.

Purpose: This study aims to compare the efficacy of various sanitizers and hot water treatment in eliminating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and natural microflora on mung bean sprouts.

Methods: Un-inoculated or inoculated sprouts were treated with acidic electrolyzed water (AEW) (75 ppm available free chlorine, ORP 1,150 mV, pH 2.8, 180 s), acidified sodium chlorite (ASC) (1200 ppm, pH 2.3, 180 s), cetylpyridinium chloride (CPC) (2%, 180 s), ozonated water (2 ppm, 180 s), trisodium phosphate (TSP) (10%, pH 12.6, 180 s) and hot water (70°C, 20 s) at room temperature. Mean values of bacterial counts were compared using ANOVA.

Results: The hot water treatment reduced by 4.2-, 4.4-, 4.8- and 4.4-log in the population of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. and natural microflora on mung bean sprouts, respectively. On the other hand, AEW, ASC, CPC, ozonated water and TSP resulted in less than 2-log reduction in the same bacterial strains. However, hot water treatment also caused detrimental impact on the color and firmness of the bean sprouts after treatment or during storage for 4 days at 4 and 25°C. Nevertheless, the present results indicate that hot water treatment could have a high potential for postharvest control measure to improve the microbiological safety of raw mung bean sprouts compared to the chemical sanitizers. Thus, this study suggests that the method needs to be further modified to better retain the physical quality of raw mung bean sprouts.

Significance: These findings may help to expand the limited pool of information on the efficacy and feasibility of decontamination treatments on sprouts.

P1-106 Fate of *Listeria monocytogenes* on Freshly Harvested Georgia-grown Cantaloupes Treated with Sanitizers

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Introduction: *Listeria monocytogenes* was implicated in a 2011 outbreak involving cantaloupes. The netted rind and the stem scar provide significant challenges for the removal of bacterial pathogens from cantaloupes.

Purpose: To compare three different sanitizers for the removal of *L. monocytogenes* from cantaloupe stem scar and netted rind and to determine if infiltration of the pathogen occurs as a result of inoculation or sanitizer treatment.

Methods: Freshly harvested cantaloupes were spot inoculated with *L. monocytogenes* at the stem scar (6 log) and netted rind (7 log). After overnight acclimation, the melons were subjected to no treatment (control) or an 8-min treatment in 200 ppm chlorine, 3 ppm chlorine dioxide, or 5 % levulinic acid and 2 % sodium dodecyl sulfate (LASDS). Cantaloupes were analyzed on the day of treatment, day 0, after storage at 4°C for 3 and 15 days, and after 8 days stored at 25°C. Two groups of melons were analyzed at each time point, one for surface *L. monocytogenes* and the other for infiltration of the pathogen into cantaloupe flesh.

Results: Decrease (-) or increase (+) in log CFU/kg values compared to control samples on day 0/day 3/day 15 (* indicates significant difference, $P < 0.05$) were -0.73/-1.09*/-3.29*; -0.20/+0.22/-0.16; -2.59*/-2.29*/-3.52* (stem scar) and +0.51/-3.29*/-3.50; +1.95/-0.94/-1.71; -2.07*/-6.21*/-2.83 (netted rind) for chlorine, chlorine dioxide, and LASDS, respectively. Infiltration of pathogen was detected via enrichment culture (detection limit 1 CFU/sample) in both treated and non-treated cantaloupe stem scar and netted rind flesh.

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Significance: Treating cantaloupes with LASDS led to greater reductions of *L. monocytogenes* on surface tissues compared to chlorine or chlorine dioxide on the day of sanitizer treatment and for short term storage. Infiltration of *L. monocytogenes* was more prevalent at the stem scar than at the netted rind and occurred with or without immersion into sanitizer solutions.

P1-107 Microbiological Evaluation of Florida Cantaloupe Packinghouses

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Introduction: Outbreaks in 2011 and 2012 were traced back to unsanitary conditions in cantaloupe packinghouses. During the 2013 cantaloupe season, FDA initiated nationwide cantaloupe packinghouse inspections with sampling components targeting *Listeria monocytogenes*, beginning in Florida.

Purpose: The purpose of this study was to evaluate the sanitary conditions of Florida cantaloupe packinghouses prior to FDA inspections.

Methods: In May 2013, five Florida cantaloupe packinghouses were visited in advance of FDA. Up to 60 swabs per facility were collected from food contact and non-contact surfaces, and water. Each sample was enumerated for total plate counts (TPC), generic *E. coli*, and coliforms, and enriched for *Listeria* spp. and *L. monocytogenes* by standard methods. Presumptive *L. monocytogenes* colonies were confirmed by amplification of the *sigB* gene by PCR and analyzed by PFGE.

Results: Environmental samples (270 swabs and 1 water sample; 172 'zone 1' food contact swabs) were collected. Average zone 1 TPC for all facilities was 6.9 ± 0.9 log CFU/swab; within facilities zone 1 TPC's ranged from 5.4 ± 1.5 (facility D) to 7.5 ± 8.3 (facility D) log CFU/swab. Coliforms and *Listeria* spp. were recovered from 36.6% and 73.2% of zone 1 swabs from all facilities samples, ranging between 27.8% (facility A) - 47.1% (facility D) and 50.0% (facility D) - 86.5.4% (facility A), respectively. *E. coli* was not recovered from any facility (limit of detection 50 CFU/swab). *Listeria monocytogenes* was recovered from 2/270 swabs (0.7%) in one facility; both food contact surfaces. PFGE patterns of the two isolates were identical. Following additional facility sanitation, *L. monocytogenes* was not detected upon retesting.

Significance: The prevalence of *L. monocytogenes* in Florida cantaloupe packinghouses was low; FDA sampling did not find any positive samples. TPC, coliforms, *E. coli* and *Listeria* spp. were not good indicators of *L. monocytogenes*.

P1-108 Efficacy of Household Sanitizers for the Reduction of *Listeria monocytogenes* and *Salmonella* s.v. Typhimurium on Cantaloupe

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Poster

Introduction: Recent outbreaks and recalls associated with cantaloupe contaminated with *Salmonella* Typhimurium and *Listeria monocytogenes* have led to an increasing need for methods consumers can use at home to clean and sanitize cantaloupe before consumption.

Purpose: Hot water and household chemicals available to consumers were tested to determine their efficacy for the reduction of *S.* Typhimurium and *L. monocytogenes* inoculated on cantaloupe surfaces.

Methods: The efficacy of hot water (82°C) and household chemicals (bleach (0.25% and 0.02% chlorine), vinegar (1% and 0.1% acetic acid), iodine (0.1%), salt (3%) and hydrogen peroxide (1% and 0.1%)) were tested for the reduction of *S.* Typhimurium and *L. monocytogenes* inoculated on the cantaloupe surface either by soaking in the solution for 5 or 10 minutes, or by first scrubbing the cantaloupe surface under running water for 1 minute prior to soaking in the solution. Bacteria were enumerated from the treated and control samples to calculate the average log reduction for each treatment. Cryo-scanning electron microscopy was performed to demonstrate that biofilms were present before treatment.

Results: Overall, scrubbing the cantaloupe surface prior to exposure to the sanitizer had a greater log reduction compared to the sanitizer alone. Hot water exposure for 5 minutes with scrubbing was the most effective at reducing *S.* Typhimurium on the cantaloupe surface (3.8 log reduction). For *L. monocytogenes*, the hot water treatment for 5 minutes both with and without scrubbing had the highest log reduction (2.5 and 2.2, respectively).

Significance: These results demonstrate consumers can use household sanitizers to reduce pathogenic bacteria on cantaloupe surfaces if they are present. However, the results also indicate it is difficult to effectively remove biofilms once they are present and emphasizes the importance of on-farm prevention to inhibit the formation of biofilms by human pathogens on cantaloupe.

P1-109 Control of the Microbial Quality of Cantaloupes via Employment of a Processing Line Incorporating Chlorine Dioxide as an Intervention Step

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Introduction: Recent listeriosis and salmonellosis multi-state outbreaks linked to consumption of cantaloupe underscore the need for effective interventions to improve microbial quality and pathogen reduction in this commodity.

Purpose: Here we investigated the microbial quality and safety of cantaloupes and the effectiveness of a commercial processing line employing a chlorine dioxide wash in improving these properties.

Methods: Determination of total plate counts (TPC) and fecal coliform counts (FCC) was performed for individual cantaloupes pre and post wash using the FDA BAM soak method, as well as for environmental samples using environmental sponges. Investigations for presence of *Listeria monocytogenes* and *Salmonella* spp. were performed by pooling soak supernatants, selective enrichments, and Modified Moore Swab bacterial concentrates, followed by testing with Neogen Reveal 2.0 lateral flow kits (LFD). Confirmation was done via selective plating, Riboprinting, biochemical testing, 16s sequencing, and real time PCR with pathogen-specific MicroSEQ assays.

Results: TPC were statistically (*t*-test, $P < 0.05$) lower for pre-wash (avg. 1.61×10^7 CFU/ml, n = 97) compared to post-wash cantaloupes (average of 5.20×10^7 CFU/ml, n = 93). In contrast, FCC were lower for post-wash (avg. 4.85×10^1) compared to pre-wash cantaloupes (avg. 2.44×10^2). No statistical differences in TPC or FCC were observed between environmental swabs samples taken upstream or downstream of the washing step (n = 23). *L. monocytogenes* was not detected; however, LFD and real time PCR results indicated the presence of *Salmonella* spp. in both pre (4 out of 10 by LFD; 3 out of 10 by PCR) and post-wash (10 out of 10 by LFD; 7 out 10 by PCR) cantaloupe pooled samples (isolates later confirmed by other methods as *Citrobacter* spp. and *Proteus* spp.).

Significance: This study supports the finding that commercial cantaloupe processing lines employing chlorine dioxide as an intervention step are not effective in improving microbial quality and safety of cantaloupes.

P1-110 Quantitative Transfer of *Listeria monocytogenes* during Mechanical Slicing and Dicing of Cantaloupe and Honeydew Melon

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Developing Scientist Competition

Introduction: The 2011 multistate outbreak of listeriosis associated with whole cantaloupe has heightened concerns regarding best commercial handling and preparation practices for melons.

Purpose: Consequently, this study aimed to quantify *Listeria monocytogenes* transfer from one inoculated cantaloupe or honeydew melon to subsequent uninoculated melons during mechanical slicing and dicing.

Methods: Retail cantaloupe and honeydew melons were washed, dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (strains M3, J22F, and J29H) to contain ~5 log CFU/cm^2, air-dried for 1h and stored at 4°C for 24h. In triplicate experiments, two cavity-cleaned inoculated melon halves were mechanically sliced with the rind (0.75 inch slices, Vollrath Redco 401N) or diced without the rind (1.0 inch dices, Nemco 55650 dicer) followed by eight uninoculated melon halves. Alternate slices (~50 to 100 g/slice) and diced melon halves as well as Kimwipe® samples from the slicer/dicer pusher and blade were homogenized in UVM, serially diluted and surface-plated with/without membrane filtration on trypticase soy agar containing 0.6% yeast extract, 0.1% esculin, and 0.05% ferric ammonium citrate to obtain black *Listeria* colonies which were counted after 24h at 37°C. Any samples negative for *Listeria* by direct plating were subsequently enriched for 48h and then streaked onto Modified Oxford Agar for confirmation.

Results: Cantaloupe and honeydew melons inoculated at 5.3 and 4.4 log CFU/cm^2, respectively, yielded *L. monocytogenes* populations of 1.9 and 1.8 CFU/g in the melon flesh after slicing. Cantaloupe inoculated at 3.1 CFU/cm^2 after rind removal yielded 2.1 CFU/g after dicing. *Listeria* populations in slices from previously uninoculated cantaloupe and honeydew melon were similar ($P > 0.05$), ranging from -0.23 to 1.24 and -0.23 to 0.34 CFU/g, respectively. The pusher and blade of the mechanical slicer and dicer were the primary contributors to *Listeria* transfer during slicing and dicing.

Significance: While the rough cantaloupe surface is far more prone to contamination than honeydew, these results demonstrated similar transfer rates to both types of melon during slicing with these findings useful in further refining risk assessment models for fresh-cut produce.

P1-111 A Surveillance Study of Fresh Cantaloupe from Indiana Farms Following 2012 *Salmonella* Outbreak

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Introduction: As a result of the 2012 *Salmonella* outbreak associated with Indiana grown cantaloupe, the Indiana State Department of Health (ISDH) started a new food safety surveillance initiative to collect fresh produce samples from Indiana wholesale produce farms and analyze them for the presence of bacteriological contamination.

Purpose: The goal is to prevent the distribution of these contaminated products to the consumer and avert foodborne illness outbreak.

Methods: Cantaloupe and watermelon samples collected for this surveillance initiative were enriched by totally submerging the whole sample into selective media and were incubated overnight. For *Salmonella* screening, aliquots were transferred and tested using BAX Q7 *Salmonella* kits. For *Listeria* spp., aliquots were transferred and tested using VIDAS LIS kits. All presumptive positive results from the screening steps were followed by culture confirmation assay recommended by the U.S FDA BAM method. Additionally, all confirmed positive samples were also confirmed with VITEK 2Comp and MADLI-Biotyper. Finally, all confirmed *Salmonella* and *Listeria monocytogenes* were further analyzed by PFGE and posted to PulseNet.

Results: Samples were collected from 5 farms in the northern part of the State and 11 farms in southern part of the State. Cantaloupes were collected during July, August, September and October 2013. A total of 76 cantaloupes and 7 environmental swabs were collected and tested for *Salmonella* spp. Another 68 cantaloupes and 54 environmental swabs were also collected and tested for *Listeria* spp.

Salmonella spp. was detected in 4 cantaloupes (5.3% of total samples). Only one *Salmonella* isolate matched the previous year outbreak. Over 16% of the samples tested for *Listeria* spp. were confirmed positive (11 samples). Among these, 3 (27%) were *Listeria monocytogenes* from cantaloupe samples. Overall, bacterial contaminants, *Salmonella* or *Listeria*, were detected from 10% of all the samples analyzed.

Significance: ISDH Food Protection Division was able to communicate with the farmers where Listeria monocytogenes was isolated and advise them to voluntarily remove the remainder of these products from commerce. In addition, this data was used by the ISDH Food Safety Farm Consultants to provide education and outreach, technical assistance and environmental assessments to the Indiana produce farmers.

P1-112 Control of *Salmonella* Cross-contamination between Intact Green Round Tomatoes in a Model Flume

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Introduction: *Salmonella* is a dangerous microorganism that has been implicated in tomato-borne outbreaks. Improper sanitizer concentrations in packinghouse flumes and dump tanks can lead to cross contamination.

Purpose: The purpose of this study was to assess the ability of hypochlorous acid to prevent cross contamination of *Salmonella*-inoculated tomatoes to uninoculated tomatoes in a model flume under clean and organic loading conditions.

Methods: Unprocessed, green, round tomatoes were inoculated with a 5-serovar cocktail of rifampicin (rif)-resistant *Salmonella* (9 log CFU/tomato), dried for 2 hr, and introduced alongside uninoculated tomatoes in a recirculating water bath containing water with simulated organic loads of 0, 500 and 4,000 ppm COD, and exposed to free chlorine levels of 0, 10 and 25 ppm. Three inoculated and three uninoculated tomatoes were removed at 0, 15, 60 and 120 s, placed into separate stomacher bags containing 100 ml buffered peptone water (BPW) + 0.1% $Na_2S_2O_3$, and vigorously rubbed/shaken to recover any *Salmonella*. One ml samples were removed from the bags, serially diluted in BPW, and pour plated in tryptic soy agar (+ rif) to enumerate any *Salmonella* present. Each experiment was performed in triplicate (n = 9).

Results: Uninoculated tomatoes were cross contaminated to a level of ca. 5 log CFU/tomato when free chlorine was absent under all loading conditions. Under 500 ppm COD organic loading, no cross contamination (<2 log CFU/tomato) was observed when 10 or 25 ppm free chlorine was present. Under a 4,000 ppm COD organic load, 10 ppm free chlorine failed to prevent cross contamination; however, no cross contamination (<2 log CFU/tomato) was observed at 25 ppm free chlorine.

Significance: These data suggest that maintaining free chlorine as low as 25 ppm is adequate to prevent cross contamination of *Salmonella* in tomato packinghouse flumes or dump tanks as long as those levels are properly maintained.

Poster

P1-113 Efficacy of Four Different Sanitizer Treatments against *Salmonella* on Smooth and Interlocking Belts during Conveyance of Diced Tomato

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Introduction: Proper design, cleaning and sanitizing of conveyor belts remains a problem for the food industry with development of biofilms on equipment surface also hindering sanitizer efficacy.

Purpose: This study aimed to assess the efficacy of four sanitizers against *Salmonella* on a custom dual belt conveyor system during conveyance of diced tomatoes.

Methods: Four kg of diced tomatoes containing avirulent *Salmonella* Typhimurium LT2 (10^7 CFU/g) were placed in an inoculation tray through which an interlocking and smooth belt (Intralox, Harahan, LA) were passed for 5 min for uniform inoculation. Five treatments - 80 ppm peroxyacetic acid (Tsunami 100, Ecolab), 80 ppm mixed peracid (Tsunami 200, Ecolab), 80 ppm chlorine at pH 6.0 (XY-12, Ecolab), 80 ppm chlorine in electrolyzed water at pH 3.0, and water (control) were continuously sprayed onto both belts (30 l/h) for 20 min. Before and after spraying, Kimwipe samples collected from selected surfaces of the conveyor belts were stomached in neutralizing buffer for 1 min and then surface-plated on trypticase soy agar containing 0.6% yeast extract, 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate with/without 0.45 µm membrane filtration to quantify *Salmonella*.

Results: For the smooth and interlocking belts, *Salmonella* reductions were greater ($P < 0.05$) using mixed peracid (6.49 and 6.76 log) and peroxyacetic acid (5.95 and 6.10 log) as compared to chlorine at pH 6.0 (3.72 and 5.70 log) and electrolyzed water (3.50 and 4.53 log) with water alone decreasing populations 1.52 and 1.48 logs, respectively. Log reductions were similar for the smooth an interlocking belts ($P > 0.05$), except for chlorine at pH 6.0 which was more efficacious for the interlocking belt.

Significance: Spray application of sanitizers is an effective means to reduce microbial cross-contamination during conveyance of diced tomato with electrolyzed water being particularly attractive due to its low cost and ease of preparation.

P1-114 Effect of Cooling Methods, pH and Temperature on *Salmonella* Survival on Inoculated Intact and Pureed Strawberries and Pureed Tomatoes

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Poster

Introduction: Postharvest strawberries are chilled to 3-4°C, usually by forced-air-cooling. Hydrocooling could ensure faster cooling, although the risk of cross-contamination for strawberries has not been evaluated. *Salmonella* survives on wounded berries, but is unable to multiply. Limited information is available on whether the inability to multiply is due to the low pH or other intrinsic factors associated with strawberries.

Purpose: This study evaluated *Salmonella* survival a) on the surface of intact hydrocooled and forced-air-cooled strawberries; b) as affected by pH, temperature and food matrix, in strawberry and tomato puree.

Methods: Intact strawberries inoculated with *Salmonella* (10^7 CFU/berry) and packed in plastic 'clamshells' (8 berries/shell) were subjected to a) forced-air-cooling at 2°C for 90 min or; b) hydrocooling in water (2-5°C) containing a sanitizer (100 or 200 ppm active chlorine) for 12 minutes. *Salmonella* population was enumerated on tryptic soy agar at 0, 7 and 8 days post-treatment. Strawberry and tomato puree (pH 3.7 and 4.6) were spiked with *Salmonella* (10^5 CFU/25 g), and incubated at 4, 10 or 25°C. *Salmonella* survival was evaluated 0, 1 and 3 days post-inoculation. Each experiment was performed in triplicate (n = 9).

Results: On day 0, *Salmonella* survival on intact, hydrocooled strawberries was reduced by > 4.0 log CFU/berry, compared to forced-air-cooled berries. *Salmonella* was below enumerable levels (1.5 log CFU/berry) on hydrocooled strawberries on days 7 and 8. When incubated at 4 and 10°C, *Salmonella* survived for 3 days, but did not multiply in either strawberry or tomato puree, regardless of the pH. When incubated at 25°C, *Salmonella* was capable of growth in both strawberry and tomato puree, when the pH was 4.7.

Significance: *Salmonella* survival on inoculated intact strawberries was reduced when subjected to hydrocooling, compared to forced-air-cooling. Food matrix did not significantly affect *Salmonella* survival or growth, whereas temperature and pH significantly affected growth.

P1-115 Influence of Temperature Differential between Green Tomatoes and Postharvest Water on *Salmonella* Internalization

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Developing Scientist Competition

Introduction: Following harvest when tomato fruits, warm with field heat, are placed into cold water (negative temperature differentials) foodborne pathogens can internalize, necessitating a 5°C temperature differential between tomato pulp and water.

Purpose: Our objective was to evaluate *Salmonella* internalization into mature green tomatoes with cooler pulp temperatures at various temperature differentials, simulating repacking operations.

Methods: Green tomatoes (21°C) were submerged into a 6 log CFU/ml, six strain *Salmonella* cocktail, maintained at 26, 24, 21, 18, or 16°C (± 5, ± 3, 0°C temperature differentials) for up to 5 minutes. To serve as positive controls, tomatoes were submerged for 30 min. Following submersion, tomatoes were surface sterilized, the stem scar and blossom end skin removed, and cores recovered. Cores were cut into three segments: an upper segment just below the stem scar (A), a middle segment (B), and a lower segment just above the blossom end (C). *Salmonella* populations in each segment were enumerated by MPN analysis following standard US FDA BAM methods. The effect of temperature differential on *Salmonella* populations in each segment was analyzed using ANOVA.

Results: *Salmonella* populations internalized into segment A at -5, -3, 0, 3, 5°C temperature differentials were < 0.08 ± 0.0, 3.5 ± 0.8, < 0.0 8± 0.0, 0.3 ± 0.3, 0.3 ± 0.4, and 0.3 ± 0.6, 0.6 ± 1.0, 0.1 ± 0.1, < 0.08 ± 0.0, < 0.08 ± 0 log MPN/segment, at 30 and 120 s respectively. No significant differences ($P \geq 0.5$) in *Salmonella* internalization into 21°C green tomatoes exist between temperature differentials at either time point. While not significant, internalized *Salmonella* populations are 0.49 log and 0.27 log MPN/segment greater at -3 and -5°C temperature differentials than those at 3 and 5°C temperature differentials after 120 s submersion.

Significance: *Salmonella* internalized into 21°C green tomatoes at low levels through the stem scar under all conditions tested; inoculum populations (6 log CFU/ml) are higher than expected under typical repacking operations where sanitizers would be used to prevent cross-contamination through water.

P1-116 *Salmonella* Newport and Typhimurium Colonization of Fruit Differs from Leaves in Various Tomato Cultivars

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Developing Scientist Competition

Introduction: Several outbreaks of *Salmonella enterica* infections have been linked to tomatoes. One cost-effective way to complement on-farm preventive measures such as Good Agricultural Practices (GAP) is to identify cultivars with inherent decreased susceptibility to *Salmonella* colonization. Although differential colonization of tomato leaves has been observed for different cultivars, fruit from various cultivars have not been assessed.

Purpose: The purpose of this study was to evaluate fruit and leaves of thirteen tomato cultivars (*Solanum lycopersicum*) with distinct phenotypes for their susceptibility to *Salmonella* epiphytic colonization.

Methods: Field-grown fruit or gnotobiotically-grown seedling leaves were spot inoculated with either a laboratory strain of *S*. Typhimurium or a tomato outbreak-associated strain of *S*. Newport. *Salmonella* were retrieved and enumerated using direct plating after 24h for fruit and 72h for seedling leaves. Differences in log CFU/unit of sample detected between levels of treatments were tested for significance using one-way ANOVA and Tukey's HSD test.

Results: Epiphytic colonization of tomato fruit by *S. enterica* was cultivar-dependent and serotype-specific, but did not necessarily correlate with seedling leaf colonization. Fruit of cultivar 'Heinz-1706' were the least colonized by *S*. Newport, and 'Mobox' by *S*. Typhimurium, while the highest populations were retrieved from fruit of 'Nyagous', 'LA4013' and 'Florida 91 VFF' ($P < 0.05$). By contrast, seedling leaves supporting the lowest populations were 'Florida 91 VFF', 'Movione' and 'Nyagous' ($P < 0.05$). The tomato outbreak strain of *S*. Newport attained higher population densities on fruit than *S*. Typhimurium ($P = 0.0002$), suggesting better adaptation to tomato fruit colonization.

Significance: These data reveal that susceptibility of tomato fruit to *Salmonella* colonization is highly variable and could be one criterion for cultivar selection for cultivation.

P1-117 Environmental Reservoirs of *Salmonella* spp. in Field and Water Samples Associated with North Carolina Tomato Production

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Introduction: *Salmonella* contamination of market tomatoes (*Solanum lycopersicum*) is one of the leading causes of produce-associated foodborne outbreaks. Current methods for on-farm control of microbial pathogens focus on general farming practices rather than crop- or pathogen-specific strategies.

Purpose: To identify and characterize *Salmonella* spp. within tomato production systems on three agriculturally diverse farms.

Methods: Environmental samples were collected during the NC tomato production season in 2012 (July – September) and 2013 (June- September) from 3 farm locations. Field (tomato fruit, blossom, leaf, weeds, soil) and water samples (n = 1010) were analyzed for *Salmonella* by enrichment using a modified BAM method as well as by real-time PCR. Isolates were serotyped and genotyped by pulse-field gel electrophoresis. Additionally, water samples were enumerated for generic *E. coli*using the IDEXX Colilert and quanti-tray 2000 system.

Results: For 2012-2013, *Salmonella* was isolated in June (10 isolates, 2013 only), July (28 isolates), August (35 isolates) and September (42 isolates); 59% (68/115) of isolates from water, 33% (38/115) from sediment, 4% (5/115) from tomato fruit, and 3% (4/115) from soil. Of the serotypes identified, 28% (32/115) were Paratyphi B (monophasic from water); 12% (14/115) were Newport (water and sediment); 7% (8/115) were Hartford (water and sediment); 4% (three sets of 5/15) were Agona, Montevideo, and Typhimurium (sediment, tomato, and water, respectively); with 1% (1/115) from Berta (sediment). These findings suggest that particular serotypes may dominate unique tomato environmental niches. Generic *E. coli*, using 235 CFU/100 ml from the proposed FSMA rules, predicted the presence or absence of *Salmonella*in water samples 81% (39/48) of the time.

Significance: This project provides baseline information on occurrence and environmental reservoirs of *Salmonella* spp. in tomatoes on diverse NC farms. This information will support science-based recommendations for tomato production practices leading to reduced microbial contamination and protection of public health.

P1-118 Evaluation of Risk Factors Affecting Transmission and Persistence of *Salmonella* spp. in Hydroponically Grown Tomatoes

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Developing Scientist Competition

Introduction: Over one million foodborne illnesses cases are attributed to *Salmonella* annually in the U.S. Greenhouse/hydroponic production of tomatoes has made a significant impact on the U.S. fresh-tomato market. There is little known about the possibility of contamination and internalization via greenhouse/hydroponic commercial production since these operations are usually considered relatively sanitary due to the closed environment.

Purpose: The objective of this study was to investigate the survival of *Salmonella* spp. in nutrient solutions used for commercial hydroponic tomato systems, as well as to determine if continuous inoculation of nutrient solution with Salmonella through a contaminated water source would lead to contamination in tomato fruits, leaves, roots and the formation of biofilms.

Methods: An avirulent strain of *Salmonella* Typhimurium was inoculated at 10^5 CFU/ml in nutrient solution tanks of hydroponic tomato systems. Inoculation occurred on day zero and every two weeks for twelve weeks. Non-inoculated tanks served as controls. On day zero and every other day post inoculation, the nutrient solution was analyzed by plating. Leaves and biofilm samples were collected on day zero and every two-weeks post inoculation. Fruit samples were collected six-weeks post inoculation. Leaf, fruit and root samples were analyzed using enrichment and plating methods. Biofilm coupons were analyzed using a tape FISH method. Typical *Salmonella* colonies observed from leaf, fruit, and root samples were confirmed by riboprinting.

Results: There was a two-log reduction of the cells two days post-initial inoculation. Reduction of cells continued over the two-week inoculation period with few cells surviving until the next inoculation period. Contamination occurred significantly in the root systems. In contrast, no contamination occurred in the leaf, fruit, and biofilm samples.

Significance: The results of the study show that while contaminated hydroponic nutrient solution or water leads to *Salmonella* contamination of tomato plant root, such an event may not pose a risk of *Salmonella* contamination of hydroponically grown tomatoes.

P1-119 Evaluation of a Most Probable Number Technique for the Simultaneous Quantification of *Salmonella* spp., *Shigella* spp. and *Listeria monocytogenes* in Saladette Tomatoes (*Lycopersicon esculentum* Mill) and Serrano Peppers

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Introduction: Several foodborne outbreaks have been linked to the consumption of tomatoes and serrano peppers and attributed to *Salmonella* spp., *Shigella* spp. or *Listeria monocytogenes*. It is important to have reliable methods for pathogen quantification in foods in order to generate quantitative data for risk assessment associated to the consumption of these foods.

Purpose: To evaluate the Most Probable Number technique for the simultaneous quantification of *Salmonella* spp., *Shigella* spp. and *Listeria monocytogenes* on saladette tomato and serrano peppers.

Methods: A cocktail of three *Salmonella* spp., three *Shigella* spp. and three *Listeria monocytogenes* strains were used to inoculate the surface of saladette tomatoes and serrano peppers. Two levels of inoculum were evaluated: approximately 0-1 (low) and 1-2 (high) log CFU/ml. Peppers were inoculated with 50 µl each, and tomato with 100 µl. Composite samples (8 peppers or 4 tomatoes) were rinsed and massaged for 2 min in 400 ml of universal pre-enrichment broth. Serial decimal dilutions were prepared for MPN: 100, 10, 1 and 0.1 ml. The isolation of pathogens was performed according to FDA-BAM for *Salmonella* and *Shigella* and the USDA-FSIS for *L. monocytogenes*. Experiments were conducted in triplicate.

Results: The three pathogenic bacteria were simultaneously recovered from the tomato surface in concentrations ranging from 0.0036 to 0.2300 MPN/ml for *Salmonella*, <0.003-11 MPN/ml, for *Shigella* and <0.003-0.15 MPN/ml for *Listeria monocytogenes*, while these pathogens were recovered from serrano peppers at concentrations ranging from <0.003 to 0.21 MPN/ml, <0.003 to 0.15 MPN/ml and <0.003-0.036 MPN/ml, respectively.

Significance: The Most Probable Number technique tested is useful for the simultaneous quantification of *Salmonella* spp., *Shigella* spp. and *Listeria monocytogenes* in saladette tomatoes and serrano pepper.

P1-120 Survival of Virulent and Mutated *Salmonella enterica* Newport and Typhimurium Strains on Tomato Plants and in Soils

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Introduction: Outbreaks of foodborne diseases caused by enteric pathogens have increasingly been associated with fresh produce, like tomatoes. *Salmonella enterica* has been reported to be the leading cause of these outbreaks in the United States. However, the survival mechanism of *Salmonella* on/in tomato plants as well as in agricultural soils is still obscure.

Purpose: To compare the survivals of different attenuated/mutated *S.* Newport (SeN) and *S.* Typhimurium (SeT) strains with their virulent wild type strains on/in tomato plants and in soils.

Methods: In this study, survival of nine *Salmonella* strains in soils (Exp. 1), and on/in tomato leaves (Exp. 2) were investigated, which include virulent strains SeN J1892 and SeT ATCC14028 and their corresponding attenuated/mutated strains N17 (SeN, Δ*tolC*::kan), T770 (SeT, pSLT-), TJSG (SeT, Δ*bcs*::kan), T0 (SeT, Δ*agfB*::kan), T1 (SeT, Δ*agfC*::kan), T2 (SeT, Δ*ompL*::kan), and T3 (SeT, Δ*yihT*::kan). In Exp. 1, *Salmonella* solution of each strain was mixed into soils collected from tomato fields to reach initial bacterial concentration of about 10^6 CFU/g. The bacterial population was tested 1, 4, 8, 15, 22, and 28 days after inoculation. In Exp. 2, inoculation was performed by dipping tomato leaves in 10^8 CFU/ml *Salmonella* solution of each strain for 10s. The bacterial population on/in leaf samples was tested 1, 7 and 14 days after inoculation.

Results: There was no significant difference among the tested *Salmonella* strains for bacterial survival in soils. The population decline rates of strains N17, T770, T2 and T3 on leaf surface were significantly higher than that of their wild type strains. Similarly, the populations of strains N17, T1, T2 and T3 in inoculated tomato leaves decreased more quickly than that of the wild type strains.

Significance: These results would benefit studies about *Salmonella* and tomato interaction, and bring clues to reduce *Salmonella* contamination on tomatoes.

P1-121 Examination of Overhead and Drip Irrigation and Chlorine Dioxide Treatment of Irrigation Water

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Introduction: Contaminated open surface water has contributed to produce outbreaks. The proposed produce rule of the Food Safety and Modernization Act emphasized understanding and reducing risk through irrigation water management.

Purpose: This study evaluated irrigation (overhead and drip) and water treatment (with or without chlorine dioxide) on pathogen presence and indicator organisms within an organic farming system.

Methods: Sampling of open surface water used for irrigation (free flowing and with sediment) was performed. Water samples were collected at the point of application in the treatment plots during four irrigation events, 4 plots per treatment (overhead untreated, drip untreated, overhead with chlorine dioxide and drip with chlorine dioxide). Samples were quantified for indicator organisms using a most probable number technique, and analyzed for *Escherichia coli* O157 and *Salmonella* presence.

Results: Results represent the first season of a two-year study. Free-flowing surface water samples were lower in generic *E. coli* (1.6 log MPN/100ml) compared to samples with sediment (2.8 log MPN/100ml). Chlorine dioxide treatment in the irrigation line significantly ($P < 0.05$) reduced generic *E. coli* (<1.3 log MPN/100ml). Treated irrigation samples were lower ($P < 0.05$) than untreated samples at the point of application. *E. coli* O157 was detected from the irrigation line prior to chlorine dioxide treatment (1/13). One sample at the point of application (1/72) was positive for *E. coli* O157 (untreated, overhead). For harvest one, lettuce outer leaves (7/10) yielded *E. coli* O157 in treated and untreated drip and untreated overhead plots; no lettuce heads were positive (0/64). For harvest two, *E. coli* O157 was detected from lettuce head samples (2/64) associated with untreated overhead and drip irrigation plots.

Significance: Use of a chlorine dioxide water treatment system appeared to reduce generic *E. coli* levels in water, but influence on generic *E. coli* levels in the soil and lettuce and prevention of pathogen contamination was unclear.

P1-122 Application of Pulsed Light (PL) to Decontaminate *Escherichia coli* O157:H7 and *Salmonella* and Preserve Quality on Raw Raspberries

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Developing Scientist Competition

Introduction: Raspberries have been associated with *Cyclospora* and norovirus outbreaks. Foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* may also contaminate raspberries because they share fecal-oral route with *Cyclospora* and norovirus. Raw raspberries cannot be washed with sanitizers due to their delicate texture. Thus, it has been a challenge to maintain the microbial safety without compromising the quality of raspberries. Novel techniques such as pulsed light (PL) may have the potential to meet the challenge.

Purpose: The purpose of this study was to investigate the potential of applying PL on pathogen decontamination and quality protection of raw raspberries.

Methods: Raw raspberries were spot inoculated with *E. coli* O157:H7 and *Salmonella*. Decontamination efficacy of PL on both pathogens was investigated after 5, 15 or 30s PL treatment. Also, survival population of pathogens was evaluated during 10 days at 4°C. Quality of the raspberries including the color, texture, total phenolic content (TPC), total anthocyanin content (TAC), total bacterial count (TBC) as well as total mold and yeast (TMY) were tested to observe the possible quality loss during 10 days of shelf life.

Results: The results showed that PL for 5, 15 or 30s was effective for *E. coli* O157:H7 (with 3.6, 3.4 and 3.9 log CFU/g reduction) and *Salmonella* (with 3.4, 4.0 and 4.5 log CFU/g reduction) decontamination on raspberries. During storage, the survival population of both pathogens on treated raspberries maintained at significantly lower levels compared with untreated ones. Color, texture, TPC and TAC of raspberries were not affected by 5 or 15s PL treatment during 10 days storage. While quality defects were observed on 30s PL treated raspberries after 5 days storage. PL also reduced TBC and TMY during shelf life.

Significance: This study suggests that PL has the potential to be used in the raspberry industry.

P1-123 Variables Affecting Virus Survival in Strawberries under High Hydrostatic Pressure

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Developing Scientist Competition

Introduction: High hydrostatic pressure processing (HPP) of food retains sensory qualities, but it is not clear if the process is effective for inactivation of viruses in contaminated produce.

Purpose: To evaluate the variables that affect virus survival in HPP treatment of contaminated strawberry mash.

Methods: Fresh strawberries were blended, degassed, inoculated with the virus surrogate MS2 coliphage (6-9 log PFU) and packed (1 ml) into a transfer pipet that was placed into a secondary bag to contain potential leaks. Using HPP pilot system Avure QFP 24L-828-S, inoculated strawberries were processed at constant temperature (14 to 40°C) and pressure (300 to 600 MPa) with 3-min holding time. To achieve constant temperature during the process while pressure increased, pre-tests were performed to appropriately adjust the initial process temperature (-3 to 21°C). A polyethylene cylindrical container enclosing all samples was used to maintain the holding temperature, which was monitored throughout the entire process. Viruses in matrices were quantified by plaque assay.

Results: MS2 in acidic berry mash and neutral tryptic soy broth (TSB) decreased by 0.43 and 3.2 logs, respectively, after 600 MPa treatment with 20°C holding temp. When berry samples were inoculated with 10% and 15% of MS2/TSB, the higher ratio of berry samples (90% berries with 10% inoculum) improved the MS2 survival by 2-fold (at 400 MPa x 20°C). Therefore, greater survival of MS2 was observed in the solid matrix compared to liquid regardless of pH. The MS2 inactivation in berry mash was more effective with higher pressures, but still less than 1 log inactivation was observed after 600 MPa x 20°C process. As the holding temperature increased, the MS2 survival generally decreased. However, ≤10% difference in the survival was shown in berries between 20°C and 40°C x 600 MPa processes.

Significance: The information is valuable for determining virus survival during HPP treatment of contaminated fruits.

P1-124 Norovirus Binding to Berries May be Independent of the Presence of HBGA-like Moieties and is Potentially Related to Interactions with Natural Microflora

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Introduction: Fresh produce, including berries and leafy greens, are commonly associated with human norovirus (HuNoV) outbreaks. HuNoVs bind to histo-blood group-like antigens (HBGAs) in foods. Little is known about the dynamics of HuNoV binding to berries, which may be ligand (HBGA) mediated and/or facilitated by the native microflora of the berry.

Purpose: To characterize HuNoV binding to berries.

Methods: Three varietals of strawberries and five of raspberries were collected and concentrates prepared. The strength of HuNoV GI.1 and GII.4 virus-like particle (VLP) binding to berry concentrates was determined by an ELISA, as was the presence of HBGA-like moieties (A, B, H, Le^a, Le^y). To isolate the natural microflora, whole berries were rinsed in PBS, the eluent plated on selective and non-selective media, and whole colony sequencing done on randomly selected colonies. Biofilm production of selected strains was quantified using a crystal violet biofilm assay.

Results: ELISA results indicated moderate binding of GI.1 and GII.4 VLPs to both berry concentrates (+/- absorbance ratio of 2.0-4.0). Very low levels of Le^a HBGA-like moieties were present in strawberries ($P < 0.05$), and A, B, H and Le^y moieties were not detected in either berry type. A total of 172 (n = 98 from raspberries; n = 74 from strawberries) bacterial isolates were sequenced, with the microbial profiles differing by berry type. Biofilm production also varied greatly, but strains of *Pantoea agglomerans*, *Pseudomonas moraviensis*, *Klebsiella oxytoca,* and *Enterobacteriaceae* spp. were the strongest biofilm producers (A_{405}> 2.2). Ongoing studies show that VLPs bind to extracted extracellular polymeric substances (EPS) from some of these strains (+/- absorbance ratio > 2.0).

Significance: HuNoV VLPs bind to berries that lack HBGA-like moieties and potentially to components of the natural microflora of berries. By identifying structural elements that bind viruses, targeted HuNoV removal and inactivation strategies can be developed for berries.

Poster

P1-125 Persistence of *Escherichia coli* on Basil in Tropical Environments

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Introduction: Climactic measures common to the tropics may impact the persistence of pathogenic *Escherichia coli*, and therefore the potential for outbreaks, particularly on crops typically eaten raw.

Purpose: To evaluate the persistence of *E. coli* as surrogate of pathogenic *E. coli* on field-inoculated basil in two distinct tropical environments.

Methods: Basil field trials were conducted at Waimanalo and Poamoho, Hawaii, representing coastal and inland tropical environments, respectively. Rifampicin (rif)-resistant *E. coli* was inoculated at concentrations of 4.5 (low) or 7.1 (high) log CFU/ml in irrigation water with or without Quadris® fungicide and Latron*B-1956 surfactant. *E. coli* was recovered from plant samples by stomaching and enumerated by plating on tryptic soy agar (TSA) + 50 µg/ml rif or filtration and plating on CHROMagar ECC+ 50 µg/ml rif. When counts fell below the limit of detection, samples were enriched in TSB + 50 µg/ml rif, followed by plating on CHROMagar ECC + 50 µg/ml rif.

Results: At Waimanalo, *E. coli* inoculated at the high level was recovered at concentrations of 5.3-5.4 log CFU/g immediately after inoculation, 4.2-4.8 log CFU/g after 3h, and 3.4-3.9 log CFU/g after 24h. *E. coli* could still be enumerated by filtration in some samples 4 weeks post-inoculation. At Poamoho, *E. coli* inoculated at low and high concentrations with fungicide and surfactant was recovered at concentrations of 2.4 and 4.2 log CFU/g immediately after inoculation, 0.75 and 2.3 log CFU/g after 3h, and 0.03 and 1.9 log CFU/g after 24h, respectively. After 4 weeks, *E. coli* was detected by enrichment in 7% (1/15) and 13% (2/15) of the samples taken from basil plants inoculated at low and high concentration, respectively.

Significance: *E. coli* populations declined more rapidly at Poamoho than Waimanalo. The slower initial decline in *E. coli* populations recorded in Waimanalo could be the result of climactic variation between the two sites.

P1-126 Survival of *Escherichia coli* in Field-inoculated Basil

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Introduction: Leafy herbs including basil have been associated with foodborne illness. The persistence of human pathogenic *Escherichia coli* on plants varies with plant species and growing conditions. Few studies have investigated the preharvest survival of *E. coli* inoculated on field-grown basil plants.

Purpose: To evaluate the survival of two environmental isolates of *E. coli* as surrogates of pathogenic *E. coli* after inoculation onto field inoculated-basil.

Methods: Lawns of rifampicin-resistant *E. coli* PTV 354 or LJH1614 were harvested from tryptic soy (TS) agar plates and diluted to a concentration of 7 log CFU/ml in milli-Q water or milli-Q water supplemented with fungicide (Quadris, Syngenta) at the recommended dose. Basil plants growing in a UC Davis research field were inoculated with a backpack sprayer. Plants were harvested at 2, 4, 6, 8, and 24h and at 1, 2, 3 and 4 weeks after inoculation. *E. coli* was recovered by stomaching and enumerated by plating onto TS agar or filtration and plating onto CHROMagar ECC both with 50 µg/ml of rifampicin. When counts were below the limit of detection, samples were enriched in TS broth with 50 µg/ml of rifampicin followed by plating on CHROMagar ECC.

Results: *E. coli* levels applied to basil plants were 5.5 and 6.5 log CFU/g in two successive trials. Populations of both *E. coli* strains declined by 2 log CFU/g within 2h of inoculation and by 3.5 to 4 log CFU after 24h. One week after inoculation, *E. coli* was below the limit of detection by plating (less than 10 CFU/g) but continued to be detected by enrichment throughout the duration of the field trial. Similar patterns of survival were observed for both strains regardless of the presence of fungicide.

Significance: *E. coli* surrogates inoculated on basil plants survived at low level for an extended period of time.

P1-127 Efficacy of Elution Solutions to Detect *Cyclospora cayetanensis, Cryptosporidium parvum,* and *Toxoplasma gondii* from Basil

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Developing Scientist Competition

Introduction: Parasitic diseases can be acquired by ingestion of contaminated raw or minimally processed fresh produce (herbs and fruits). The sensitivity of methods used to detect parasites on fresh produce depends in part on the efficacy of wash/elution solutions in recovering them from suspect samples.

Purpose: Identify the efficacy of elution solutions to detect *Cyclospora, Cryptosporidium*, and *Toxoplasma* oocysts from basil.

Methods: In this study, six wash solutions (sterile E-Pure water, 3% levulinic acid-3% sodium dodecyl sulfate, 1 M glycine, 0.1 M phosphate buffered saline, 0.1% Alconox®, and 1% HCl-pepsin) were evaluated for their effectiveness in recovering *Cyclospora cayetanensis, Cryptosporidium parvum,* and *Toxoplasma gondii* from basil. One hundred and 1,000 oocysts of these parasites were inoculated on adaxial surfaces of 25 g of basil leaves placed in stomacher bags, and stored for 1 h at 21°C and 24 h at 4°C. The leaves were hand washed in each wash solution for 1 min. DNA was extracted from the wash solutions and amplified using PCR for the detection of all parasites.

Results: Oocysts inoculated at a concentration of 1,000 oocysts/25 g of basil were detected in all wash solutions. At an inoculum concentration of 100 oocysts/25 g, oocysts were detected in 18.5 to 92.6% of the wash solutions. The lowest variability in recovering oocysts from basil inoculated with 100 oocysts was observed in 1% HCl-pepsin wash solution. Oocyst recovery rates were higher at 1 h compared to 24 h post-inoculation.

Significance: Unlike most bacteria, parasites cannot be enriched; therefore, an optimal recovery process for oocysts from suspected foods is critical. Observations in this study provide guidance concerning the selection of wash solutions giving highest retrieval of parasites oocysts.

P1-128 Effects of Pyrolysis Char from Various Feed Stocks on Survivability of *Salmonella enterica* and *Eschericha coli* O157:H7 in Agriculture Soil

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Introduction: Control of foodborne pathogenic bacteria in agricultural soils is a major concern for vegetable growers. Pathogenic bacteria can enter soil through a variety of vectors including; manure used for fertilizer, contaminated water, animals and farmers.

Purpose: A technology has surfaced in recent years through which waste biomass can be converted into condensable and non-condensable gases and char. This char contains the minerals and carbon of the feed stock, making it not only suitable as a fertilizer but as a method of carbon sequestration.

Methods: For this experiment, 3 feed stocks were chosen; pelletized hardwood, pelletized switch grass, and switch grass. Char was produced by pyrolysis at 500°C for <1 second (fast) or 1 hour (slow). Soil used was a mixture of soil, sand, vermiculite and turface in a ratio of 0.75:1:1:0.75 (SSVT). 100 grams of SSVT was inoculated with 6 ml of a cocktail consisting of either *S. enterica* or *E. coli* O157:H7. After inoculation, char was added to the SSVT at application rates of 1, 3 or 5%. SSVT, inoculum and char were thoroughly mixed by hand for 30 seconds. A 1 gram sample was taken immediately following mixing and then every 2 weeks for a total of 12 sampling times.

Results: At the initial sampling there was a 1-log decrease in *Salmonella* caused by the switch grass pellet slow pyrolysis char ($P = 0.0061$) and a 2-log decrease in *E. coli* ($P = 0.0483$). At subsequent sampling times significant decreases in both *Salmonella* and *E. coli* caused by all pyrolysis chars ($P < 0.05$).

Significance: These results indicate a strong potential for pyrolysis char as a soil amendment in regard to reduction of *S. enterica* and *E. coli* in agriculture type soils. Further research is needed to determine the effects of pyrolysis char on plant growth and pathogenic bacteria.

P1-129 Irrigation, Manure, and Soil Type Influences on Survival and Persistence of Non-pathogenic *E. coli* and *E. coli* O157:H7 in a Greenhouse Environment

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Developing Scientist Competition

Introduction: Application of raw animal manure to enhance soil productivity may introduce enteric pathogens that contaminate produce, but the effects of soil type and irrigation on pathogen persistence are unclear.

Purpose: To investigate 1) survival and persistence of non-pathogenic *Escherichia coli* (*Ec*) and attenuated *E. coli* O157:H7 (attO157) before and 24h after irrigation events in silt loam soil (SL) unamended (UN), amended with poultry litter (PL) or horse manure (HM) and 2) to compare survival of *Ec* and attO157 in manure-amended SL and clay loam soil (CL).

Methods: Twenty-four large pots (2552.5 cm^3), half containing either SL or CL in a greenhouse, were amended with PL, HM, or UN (n = 4 per treatment). All soils were sprayed with 7.2 x 10^8 CFU/m^2 of an inocula containing three *Ec* and two attO157 strains, all rifampicin-resistant. SL treatments were sampled on days 6, 13, 27 and 55 post-inoculation (dpi), and then both SL and CL treatments were irrigated. All samples were collected 24h after each irrigation. *E. coli* populations were determined by direct plating and/or mini-MPN.

Results: Populations of *Ec* increased by 3.11, 1.07, and 1.20 log CFU/g on days 14, 28, and 56, respectively, while attO157 populations increased by 3.2, 0.97 and 0.48 log CFU/g on these same days, after irrigation of PL-amended SL. *E. coli* populations in UN-SL increased by 0.84 log CFU/g after irrigation on day 28, but decreased 0.15 log CFU/g after irrigation on day 56. Overall, all *E. coli* populations declined more rapidly in unamended and HM-amended SL and CL than in these same soils amended with PL.

Significance: The resuscitation of *E. coli* cells was enhanced by irrigation of PL-SL even 56 dpi, in contrast to UN and HM amendment. Consideration of these findings will contribute to development of guidelines for produce safety relative to PL and other soil amendments.

P1-130 Survival of *Escherichia coli* and Attenuated *E. coli* O157:H7 in Manure-amended Soils in the Delmarva Peninsula as Influenced by Winter Weather Conditions

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Introduction: Annual fertilization of Delmarva Peninsula croplands with animal manures can increase the risk of contamination of fresh produce by pathogens present in manure.

Purpose: To investigate 1) survival of non-pathogenic *Escherichia coli* (*Ec*) and attenuated *E. coli* O157:H7 (attO157) in soils amended with poultry litter (PL) or horse manure (HM) from fall/winter 2013/2014.

Methods: Field plots (Othello soil) were amended with PL or HM. An inoculum containing equal numbers of three and two rifampicin-resistant (Rif^R) strains of *Ec* and attO157-Rif^R strains, respectively was sprayed onto soils at low 6.73 x 10^6 CFU/m^2 (LC) or high 7.2 x 10^8 CFU/m^2 (HC) concentrations, and manure was either surface-applied or tilled into soil. Soil samples were collected between 0-91 days post-inoculation (dpi), and *Ec* and attO157 populations were determined by direct plating and/or mini-MPN.

Results: Populations declined more rapidly in unamended soil than in soils amended with HM and PL. In unamended soil at HC, *Ec* concentrations declined by 2.1 and 2.4 log CFU/g in surface and core samples 91 dpi, respectively. When *Ec* was applied at LC, populations in surface and core samples declined by 2.73 and 1.39 log CFU/g, respectively, over 91 dpi, in unamended soils. A similar trend was observed in soils amended with HM and PL. At 7 dpi, populations of *EC* and attO157 in LC in surface-applied unamended, PL and HM soils increased by > 1 log CFU/g, corresponding to rain events over the same period. Generally, *E. coli* populations persisted longer in soils amended with PL than with HM and were greater in surface than in core samples.

Significance: Manure type, method of application, and winter environmental factors influenced survival of *E. coli* in soil. Both *Ec* and attO157 survived longer in manure-amended soils compared to unamended soils, indicating the effect manure has in extending survival of bacteria in soils.

P1-131 The Effect of Manure Application Method on the Persistence of *Eschericha coli* in Manure-amended Soils in Southeastern Pennsylvania

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Introduction: Animal manure, used as fertilizer, may introduce enteric pathogens to crops in agricultural fields. FDA proposes a 270-day interval between application of manure and harvest of produce crops.

Purpose: The persistence of *Escherichia coli* in manure-amended soils in Pennsylvania and its transfer to growing spinach plants was evaluated.

Methods: Inoculum containing equal amounts of attenuated, rifampicin-resistant *E. coli* O157:H7 (*attO157*) and non-pathogenic *E. coli* (*gEc*) was spray-applied at low (LP: 3.9 log CFU/m^2) or high (HP: 6.4 log CFU/m^2) populations to 2 m^2 plots amended with poultry litter (PL), horse manure (HM), or

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no manure (NM). Manure was either surface-applied or tilled into the soil; spinach was seeded in high PL 7-dpi (days post-inoculation). Surface or core samples of soils were collected to 56-dpi. *E. coli* populations (CFU or MPN/g dry weight (gdw)) in soils or from spinach was determined through 56-dpi by direct plating or by mini-MPN.

Results: Populations of *gEc* declined by 1.1–2.1 and 0.1-2.1 log CFU/gdw in LP and HP unamended plots, respectively, at 56-dpi; however, LP and HP of *gEc* did not decline in soils amended with PL as in HM-amended soils. In all manure-amended soils, *gEC* populations in core samples were >1 log CFU/gdw than surface samples on day 0. In PL plots containing HP, *gEc* populations were 6.6, 5.0, and 3.1 log CFU/gdw on 7, 28, and 56-dpi, respectively, in core samples. *gEC* recovered from spinach plants in HP PL plots were > 6, 3.2, and 0.6 log MPN/gdw at 28-, 46-, and 56-dpi, respectively. In all plots, LP and HP of *attO157*declined rapidly by day 3 and none was recovered from spinach planted in PL soils on 7 dpi.

Significance: *E. coli* was transferred to spinach when planted in PL-amended soils containing high populations, and persisted for longer durations in PL compared to HM or NM soils.

P1-132 Evaluation of Non-pathogenic *Escherichia coli* Isolates to be Used in Field Trials Examining the Persistence of Pathogens in Untreated Soil Amendments

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Introduction: The proposed FDA "Produce Rule," part of the Food Safety Modernization Act (FSMA), states that untreated soil amendments (UTSA) must be applied to agricultural fields 270 days before harvest of produce crops to minimize pathogen transfer. A framework document which evaluated experimental designs was developed for groups intending to apply for an evidence-based variance from this proposed rule. This framework recommends use of a non-pathogenic bacterium with a stable detectable phenotype and history of use in field studies as a surrogate for a pathogen compared to strain(s) with only laboratory data.

Purpose: The purpose of this study was to examine the longitudinal persistence of three non-pathogenic rifampicin-resistant *Escherichia coli* strains (TVS 353,354,355), originally isolated from agricultural environments, in various manure-amended soils to determine their suitability for use as non-pathogenic surrogates for *E. coli* O157:H7.

Methods: An inoculum containing equal populations of each of the three *E. coli* strains was applied to poultry litter-, dairy manure-, and horse manure-amended soils at two different sites (Maryland and Pennsylvania) in five separate seasons. *E. coli* were isolated from amended soils between 114 - 150 days post-inoculation. DNA was extracted and amplified by BOX-PCR to create unique banding patterns by gel electrophoresis to identify specific *E. coli*.

Results: Overall, 141 *E. coli* isolates were identified from five different field studies: 105 (74%) were TVS 355, 28 (20%) were TVS 354, and 8 (6%) were TVS 353. In four of the five studies evaluated, TVS 355 was the *E. coli* isolate isolated most frequently on the last day of the field study.

Significance: The increased prevalence of TVS 355 compared to the other *E. coli* isolates evaluated displays its extended persistence in manure-amended soils, and indicates that TVS 355 may be an appropriate non-pathogenic *E. coli* isolate to use in variance request and other UTSA field /soil research studies.

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P1-133 Efficacy of Two Hand-hygiene Methods to Reduce Organic Matter and Fecal Contamination on Farmworker Hands during Harvest

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Introduction: To prevent produce-related outbreaks, it is imperative to identify the risk factors for produce contamination on farms. In a previous study, fecal contamination was quantified on over 200 matched samples of produce and farmworker hands. Concentrations and prevalence of fecal indicator bacteria correlated between matched produce and hand samples.

Purpose: To evaluate two hand-hygiene methods to assess their ability to reduce dirtiness and indicators of fecal contamination on farmworker hands during harvest.

Methods: 159 farmworkers were recruited at two farms. Hand washing with a foam cleanser, and the SaniTwice method using an ethanol-based hand sanitizer gel were compared. The intervention groups were trained in their hygiene method, but not the control group. All groups harvested produce for 30 minutes before sampling (control group) or intervention. The intervention groups practiced hand-hygiene, and then gave hand rinse samples either immediately, or after continuing to harvest produce post-hand-hygiene. Absorbance of the hand rinsate was measured to quantify organic matter, and *E. coli*, fecal coliforms, and *Enterococcus* were enumerated as indicators of fecal contamination.

Results: The foam cleanser group had less organic matter than the method group, and both had less than the control group ($P < 0.05$).

E. coli was non-detectable in almost all groups. The control group had, on average, greater than 3 log fecal coliforms and 4 log *Enterococcus* per hand. The foam cleanser group had levels of microorganisms that were not significantly different. The method group had approximately one log fewer fecal coliforms and enterococcus per hand than the control group immediately after practicing hand-hygiene ($P < 0.05$); however, after 30 minutes harvesting, levels of microorganisms were not different from the control group.

Significance: The method is superior to foam cleanser in reducing both dirtiness and fecal indicator bacteria, and may be an appropriate hand-hygiene method when water is not available.

P1-134 A Longitudinal Comparison of *Eschericha coli*, *Salmonella* and Enteric Viruses on Wildlife Feces to Evaluate Potential Microbial Niches

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Developing Scientist Competition

Introduction: Animals may contribute to pre-harvest contamination of produce by application of raw manure as fertilizer as well as their presence in the field.

Purpose: Our objective was to assess differences in persistence of *Salmonella*, *Escherichia coli*, and viral pathogens within fecal samples collected from 7 different birds and mammals.

Methods: Manure samples were obtained fresh from animals on or near the CANR Farm (Newark, DE). Samples (25g) were inoculated with 10^6 CFU/g or 10^4 CFU/g rifampicin-resistant *Salmonella* (6), *E. coli* (5), or a viral cocktail containing hepatitis A virus, murine norovirus, and Tulane virus.

Samples were assessed after incubation at 20°C over 12 months. Bacterial samples were enumerated on TSA containing 80 µg/ml Rifampicin and 50 µg/ml Cycloheximide. Viral samples were frozen at 4°C, RNA extracted and viral genomes detected by qRT-PCR.

Results: Initial *E. coli* growth was observed in deer, waterfowl and cattle feces with highest concentrations at 15 days post-inoculation (dpi) of 7.50 log CFU/g. Following this, bacteria declined in these fecal samples and by 243 dpi concentrations of 4.93 log CFU/g cattle feces and 0.5 log CFU/g deer feces were detected. Bacteria in high and low inoculum samples were at equivalent levels within 30-45 dpi. Bacteria in waterfowl feces were found to be below the detection limit (1.5 CFU/g) in all samples at 56 dpi. Initial *Salmonella* growth was noted in all fecal types except for chicken, in which samples were below the detection limit and negative for both *E. coli* and *Salmonella* following sample enrichment. At 7 and 28 dpi, bacteria in raccoon and pig feces were below the detection limit, respectively. After 10 months, bacteria continue to be detectable in fecal samples from deer, sheep, and cattle. Further analysis continues on the virus samples.

Significance: As inferred from epidemiological evidence, cattle and deer feces were the most suitable for long-term persistence of *E. coli* and *Salmonella*, supporting growth and survival over ten months.

P1-135 Investigating the Effect of *Salmonella* Biocontrol Agent *Paenibacillus alvei* and Poultry Litter Soil Amendment on the Eastern Shore Tomato Microbiome

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Developing Scientist Competition

Introduction: Through laboratory, growth chamber, and field studies, a strain of *Paenibacillus alvei,* TS-15, has demonstrated potential to decrease the incidence of foodborne pathogen *Salmonella enterica* in tomato plants. Although TS-15 was isolated from agricultural soil, when applied to the field its concentration will be magnified in the environment. For responsible management of this biocontrol, it is important to understand how intensifying its presence in tomato fields under various soil management practices could affect resident microbial assemblages in the tomato phyllosphere and rhizosphere.

Purpose: The purpose of this study was to investigate the influence of biocontrol application with or without chicken litter soil amendment on the microbial ecology and foodborne pathogen risk of an eastern shore tomato field.

Methods: Tomatoes were field-grown with treatments including chicken litter soil amendment and TS-15 application. Microbial communities collected from roots and blossoms were described by 16S sequencing, performed by Illumina MiSeq and analyzed using QIIME. Microbiological methods including direct plating and enrichment were used to screen for *Escherichia coli, Salmonella*, and *Enterococcus* spp. on tomato plant organs throughout the season.

Results: Principal component analysis showed that tomato root and blossom bacterial communities differed in phylogenetic composition. Biocontrol treatment had no effect on blossom and root bacterial communities, while chicken litter soil amendment resulted in separate clustering for both roots and blossoms. There were no differences in *E. coli*, *Salmonella*, or *Enterococcus* levels between treatments ($P > 0.05$).

Significance: The data suggest that the application of TS-15 does not impact root or blossom bacterial communities associated with tomato plants, indicating that its use as a food safety biocontrol will not have negative effects on microbial processes integral to plant health. Chicken litter treatment could have implications for food safety; the microbial makeup of soil amendments may influence microbial diversity and function throughout the plant.

P1-136 Isolation and Characterization of *Salmonella* from North Florida Surface Waters

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Introduction: *Salmonella*-contaminated surface waters may lead to preharvest produce contamination if water contacts the harvestable portion of the crop.

Purpose: The aim of this study was to understand the environmental distribution and diversity of *Salmonella* in North Florida surface waters and the association of environmental factors with *Salmonella* presence.

Methods: Surface water samples (10 l) were collected monthly for 12 months (11/2011-10/2012) from six sites located near agricultural lands in North Florida. Samples were analysed for physical and chemical characteristics; enumerated for total aerobic plate count (TPC), total coliforms, generic *Escherichia coli* and *Salmonella* by standard FDA-BAM methods. *Salmonella* isolates were characterized by serotyping, PFGE, and antimicrobial resistance. A previously published model for the probability of enumerating *Salmonella* as a function of *E. coli* concentration in Central Florida surface waters was evaluated for its applicability to this data.

Results: *Salmonella* was isolated from 21% (15/72) of water samples; 20 representative isolates (one isolate per site, per month, per serotype) were recovered. Serotypes included *Salmonella* Inverness, Muenchen, Saintpaul, IV_40:z4, z24:-, Florida, Hartford, and Anatum. One isolate was untypeable. Positive samples were evenly distributed across the sample sites. No positive samples were detected in the spring (March-May). Environmental factors (TPC, coliforms, *E. coli*, temperature, pH, ORP, turbidity) did not predict *Salmonella* presence/absence. Results collected here could not validate the previously published predictive model due to the low prevalence of *Salmonella* detected; the reported trend where increasing concentrations of *E. coli* correlated with increasing probabilities of detecting a *Salmonella*-positive sample was also observed. Proposed FSMA preharvest fruit-contact surface water standards require <235 MPN generic *E. coli*/100 ml in a single sample; 20% (14/72) of water samples (from 5/6 sources) were above this limit.

Significance: *Salmonella* populations are present in North Florida surface waters and cannot be predicted by the environmental factors evaluated or a previously published model.

P1-137 Microbial Quality of Surface Agricultural Water in Central Florida

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Developing Scientist Competition

Introduction: Current recommendations for microbiological quality of water contacting the harvestable portion of produce include, generic *Escherichia coli* populations ≤ 235 (2.4 log) MPN/100 ml in a single sample and a geometric mean (n = 5) ≤126 (2.1 log) MPN /100 ml, with testing occurring weekly for open surface waters.

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Purpose: The purpose of this study is to evaluate the influence of events that may result in episodic contamination of agricultural pond water.

Methods: Water samples (500 ml) from six ponds in Central Florida were collected weekly, November - May, weekly and every other day after rain exceeding 2.0 cm within 24h, and freeze protection. Samples were collected 20 cm below surface within a 304.8 cm radius of the water intake. Microbial populations (total coliforms, generic *Escherichia coli*, and *Enterococci*) were enumerated by MPN. Environmental variables measured included, water and ambient temperature, pH, conductivity, oxidation and reduction potential, and turbidity; observed animal activity was noted.

Results: *Escherichia coli* populations were ≤ 2.4 log MPN/100 ml in 247/276 (89.5 %) and geometric means were ≤ 2.1 log MPN/100 ml in 252/276 (91.3%) of samples. Following rain events, populations increased up to 4.2 log CFU/100 ml from the previous sampling. Populations of coliforms and *Enterococci* ranged from log 2 to 8 and 1 to 5, respectively. Microbial populations varied within ponds varied up to 4 log MPN/100 ml between sample points. Pearson product moment correlation coefficients (r) among microbial indicators were above 0.61 ($P < 0.0001$). Microbial indicators were not correlated with environmental variables, except pH ($P < 0.0001$). Animal activity during sampling included amphibians, fish and birds.

Significance: Surface waters tested in Central Florida met the current recommendations for microbial water quality 85.5% (236/276) of the time. Environmental factors may change the microbial quality of surface water; understanding the effects will allow targeted sampling of surface water to reduce food safety risks.

P1-138 Low Concentration of *Salmonella* in Farm Ponds and Irrigation Distribution Systems Used for Mixed Produce Production in Southern Georgia

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Introduction: Because irrigation water has been shown to be a vector for the contamination of fresh produce by pathogenic bacteria, the proposed produce safety rule under the Food Safety Modernization Act (FSMA) requires that agricultural water must be safe and of adequate sanitary quality for its intended use. These requirements may be challenging for farmers using surface water sources such as constructed farm ponds.

Purpose: The purpose of this study was to assess the presence and concentration of *Salmonella* and indicator bacteria in irrigation water exiting different distribution systems on a mixed produce farm growing tomatoes, squash, peppers, eggplant, cantaloupe, and leafy greens in southern Georgia.

Methods: *Salmonella* and generic *Escherichia coli* concentrations (CFU/100 ml) were monitored in 2 irrigation ponds supplying drip, center pivot, and solid set sprinkler systems and one deep groundwater well feeding a drip system. Samples were collected during three growing seasons in 2012-2013. *Salmonella* isolates were subtyped by pulsed-field gel electrophoresis (PFGE).

Results: *Salmonella* was found in water from 15/74 (20%) pond, 12/49 (24%) pivot, 1/24 (4%) solid set sprinkler, 7/48 (15%) drip line (start) and 11/48 (23%) drip line (end) samples. The mean *Salmonella* concentration was 0.3 MPN/100 ml (range 0.1 – 1.8 MPN/100 ml) in positive samples. No *Salmonella* or generic *E. coli* were detected in samples collected from the well (n = 80) or drip system (n = 24) fed from the well. *E. coli* concentrations in samples from irrigation systems containing *Salmonella* were below the proposed FSMA threshold of 235 CFU/ 100 ml. Indistinguishable *Salmonella* subtypes were found in pond-pivot and pond-drip samples collected on the same day and location.

Significance: Knowledge resulting from this project will allow vegetable producers that rely on untreated surface sources of irrigation water to effectively address new rules the FDA may implement on safe agricultural water.

P1-139 The Occurrence of Bacterial and Viral Foodborne Pathogens in Irrigation Waters in Southern Arizona

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Introduction: Numerous outbreaks related to produce have been attributed to *Salmonella*, *Escherichia coli* O157:H7, human norovirus (NoV), and hepatitis A virus (HAV).

Purpose: To investigate the role of irrigation waters as potential sources of produce contamination by foodborne pathogens.

Methods: Fifteen samples were collected from irrigation canals in Yuma and Maricopa Counties in Arizona, for four months during the leafy greens growing (December-March) and non-growing (July-October) seasons over two years (236 total); 1-l grab samples were collected for bacteria and 100-l samples using NanoCeram® filters for viruses. Filter eluates were further concentrated using centrifugal ultrafiltration. HAV, NoV, Aichiviruses (AiV), and pepper mild mottle virus (PMMV) were identified using qPCR.

Results: All samples were negative for NoV, HAV, and AiV; however, 28.6% of samples had PMMV present. A majority of samples tested positive by Colilert® for coliforms (100%) and *E. coli* (88.1%). None of the sites exceeded current standards of 126 *E. coli*/100ml (geometric mean; ≥5 samples). *Salmonella* (14.0% water, 22.6% sediment samples) and/or *E. coli* (9.3% water, 8.1% sediment samples) were isolated using enrichment/selection methods. *Salmonella* were confirmed via PCR for *invA* and *himA* genes; no *E. coli* were positive for O157 antigen or virulence genes (*stx1/stx2/eae*). No overall correlations were found between any water quality parameters collected, the presence of bacteria, and the presence of PMMV. The average numbers of PMMV per site in Yuma (but not Maricopa) could be weakly correlated with the occurrence of coliforms ($r = 0.69$), *E. coli* in water ($r = 0.72$ and $r = 0.57$ for Colilert® and enrichment/selection, respectively), and *Salmonella* in water ($r = 0.51$).

Significance: Although no pathogenic viruses were found, the presence of PMMV suggests human fecal contamination. Also, although it appears that these irrigation waters are generally of high quality, pathogens are present on occasion. Thus, the water may be a potential source of crop contamination.

P1-140 Evaluation of Indicator *Escherichia coli*, Fecal Coliforms, *E. coli* O157 and *Salmonella* spp. in Surface Waters of the Southwest Desert Canal Network

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Introduction: Irrigation canals are an important water delivery system for the Southwest desert farming region. Industry guidance and proposed produce safety regulations have recommended that stakeholders use fecal indicator bacteria as a proxy for deleterious water quality. It is unclear whether larger volumes or targeted sampling would improve monitoring strategies for canal irrigation systems.

Purpose: The purpose of this study is to determine the relationship between fecal indicator bacteria and pathogens in irrigation water supplies and whether large volume sampling for pathogens is more informative for microbial water quality.

Methods: Seven sites were sampled monthly at overpasses along canal networks in the Southwest US. Using ultrafiltration (20 l) samples were analyzed for indicator *E. coli* and fecal coliforms, *Salmonella* and *E. coli* O157:H7. Environmental and water quality parameters were collected at each site. Sampling for 2013 was conducted from June to Novemberl; sampling for 2014 will be performed from January to April.

Results: A total of 76 water samples were collected over 6 sampling events. To date, *Salmonella* was found in 29 screened samples while no samples tested positive for *E. coli* O157:H7. The presence of *Salmonella* was not associated statistically with an increase in indicator bacteria (*E. coli* and fecal coliforms). Average indicator *E. coli* counts (CFU/100 ml) were well below the standard of 235 CFU/100 ml ($\mu = 46.1$, $\sigma = 82.4$) and did not vary significantly ($P > 0.05$) across the sampling period. Fecal coliforms varied across time and space (event and site).

Significance: The water quality standard adopted by the produce industry and slated for inclusion in the Food Safety Modernization Act for the safe application of irrigation water is 235 CFU/100 ml for *E. coli*. Our data indicate that the presence of pathogens in irrigation water supplies is not predicted by an excess in the current water quality standard in a major southwestern desert produce production region.

P1-141 Examination of Indicator Organism Levels in Open Surface Water Sources Used for Irrigation and Overhead Cooling of Apples in Washington

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Introduction: The currently proposed produce rule of the Food Safety Modernization Act provides numerical standards for generic *Escherichia coli* levels associated with agricultural water used during growing activities.

Purpose: The study evaluated fecal coliform and generic *E. coli* levels in open surface waters used for irrigation and overhead cooling of apples in Washington.

Methods: Sampling locations (24) in three regions of central Washington were identified and selected sampling sites examined variation in water quality from the water source, holding and final water delivery method. Multiple samples were collected from sampling sites to evaluate consistency between bacterial levels at multiple points within a site. Water samples were collected monthly for at least three months (June – August) with 98 total sampling sites. Water samples were tested for fecal coliforms and generic *E. coli* using the Most Probable Number method and IDEXX Colilert®-18, Quanti-Tray®/2000 system.

Results: For 293 samples evaluated using the MPN method, approximately 15 samples (5.1%) exceeded the currently proposed FDA standard for any single sample (235 MPN/100ml generic *E. coli*). The southern region had the highest number of samples exceeding the proposed single sample standard (10) followed by central (4) and northern (1) regions. Sites exceeding the single sample standard were evenly distributed over time (5 each month), and were collected from canals/laterals (7), ponds (6), river (1) and a water box (1). Only one of 98 sampling sites (1%) exceeded the proposed rolling geometric mean standard (126 MPN/100ml). Results using IDEXX Colilert®-18 system did not always align with MPN values; however, biological variation likely contributed to this observation.

Significance: Understanding trends in generic *E. coli* levels associated with individual, open surface water bodies and water used for direct produce contact can assist growers, scientists and regulators in determining optimal risk management strategies.

P1-142 Prevalence of Shiga Toxin-producing *Escherichia coli* in Irrigation Waters and Fresh Produce in British Columbia, Canada

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Developing Scientist Competition

Introduction: Irrigation water is a potential risk factor in the transmission of Shiga toxin-producing *Escherichia coli* (STEC) to fruits and vegetables during production. However, little is known about geographical and temporal variability in the prevalence of STEC in irrigation water and on field-grown produce.

Purpose: To determine the seasonal prevalence rates and characteristics of STEC in irrigation waters and on fresh produce from three growing regions in British Columbia, Canada.

Methods: Water samples were collected monthly from irrigation ditches and streams in 20 sites located in three distinct watersheds (R, CA, LS) in British Columbia, Canada. Locally-grown leafy vegetables and berry fruits were obtained from seven farm markets during the summer months. A novel hydrophobic grid membrane filtration (HGMF) - Stx immunoblot method was used to detect and isolate STEC among the background microflora in samples of water and produce rinses tested without enrichment. Presumptive STEC isolates were tested for virulence genes (*eaeA*, *hlyA*, stx_1 and stx_2) by multiplex PCR and confirmed isolates were serotyped.

Results: STEC were not recovered from 105 samples of produce (79 vegetable, 26 berry). In contrast, STEC were isolated from 63 of 330 (19.1%) water samples. Prevalence rates varied with season, ranging from 34.2% to 15.7% and 13.3%, in winter, spring/summer and fall months, respectively. Isolates recovered from water included 9 (4.2%) of serotypes O157:H7 or O157:NM, 29 (13.6%) of non-O157 "priority" serogroups O26, O103 and O111, and 176 (82.2%) of 31 other serotypes. The 214 STEC isolates were randomly distributed among 11 distinct virulence gene patterns, providing evidence of considerable genotypic diversity in environmental STEC populations.

Significance: STEC were not recovered from fresh produce despite their frequent recovery in irrigation waters. Additional research is needed to clarify the role of biotic and abiotic factors on the transmission of STEC to field-grown produce.

P1-143 Role of Washing Water Parameters on Inactivation of *Salmonella* during the Disinfection Step of Minimally Processed Vegetables

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Introduction: The efficacy of the disinfection process of minimally processed vegetables (MPV) and consequently the quality and safety of the final product are strongly dependent on the washing water parameters.

Purpose: This study aimed at assessing the influence of washing water parameters (pH, organic load, temperature, chlorine concentration and time of contact) on inactivation of *Salmonella* during the disinfection step of minimally processed lettuce and carrots, mimicking practices used in MPV processing plants in Sao Paulo, Brazil.

Methods: Experimentally contaminated minimally processed lettuce (1 kg) or carrots (1 kg) were added to 8 l of water presenting pH 5.6 and 8.0, organic load 2.3 and 13.5 mg/l and chlorine 50 and 250 mg/l. The temperature of the water was 10 and 25°C and the time of contact was 2 and 20 min.

Contamination was obtained by immersion for 30 min in water containing *Salmonella* (10^6CFU/ml). The tested parameters corresponded to the lowest and highest values encountered in the surveyed MPV processing plants.

Results: The combination of pH 8.0, organic load 2.3 mg/l, chlorine 250 mg/l, time of contact 20 min and temperature 10°C resulted in the highest reduction in the populations of *Salmonella*: 2.0 and 2.8 log for lettuce and carrot, respectively. Conversely, the combination of pH 5.6, organic load 13.5 mg/l, chlorine concentration 50 mg/l for 2 min at 25°C resulted in the lowest reduction: 0.5 and 1.1 log for lettuce and carrot, respectively. Factorial ANOVA test indicated that among the tested parameters, only chlorine concentration was significant ($P<0.05$) for reduction of *Salmonella* during the disinfection step.

Significance: Chlorine concentration is the most important parameter for reduction of *Salmonella* during the disinfection step of minimally processed lettuce and carrots, while pH, organic load, temperature and time of contact were less relevant.

Acknowledgements: FAPESP, CNPq and CAPES

P1-144 The Effect of Water Velocity on *Escherichia coli* O157:H7 Transfer from Inoculated Lettuce to Wash Water in a Closed Pipe System

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Developing Scientist Competition

Introduction: Cross-contamination of fresh-cut leafy greens during commercial flume washing continues to pose major food safety concerns for the industry.

Purpose: This study aimed to quantify *Escherichia coli* O157:H7 transfer from lettuce to water at different velocities in a closed pipe system.

Methods: One ml of a 4-strain nontoxigenic *E. coli* O157:H7 cocktail containing 8.1 log CFU/ml, or 5 x 5 cm pieces of iceberg lettuce dip-inoculated to contain *E. coli* O157:H7 at ~7 log CFU/g, were inserted into continuously flowing water pumped through a 10-cm dia pipe at 0.0756, 0.108 and 0.189 l/s. Water and lettuce samples were collected at 5- or 10-sec intervals for 2 min and quantitatively examined for *E. coli* by plating appropriate dilutions with/without membrane filtration on TSAYE with ampicillin. Data from triplicate experiments were assessed for significance using the Kenward-Roger correction.

Results: In the water study, cumulative populations of *E. coli* O157:H7 recovered were inversely related to flow rate. Using inoculated lettuce, *E. coli* O157:H7 populations in the water were significantly higher at a flow rate of 0.189 l/s (2.5 log CFU/ml) ($P < 0.05$) compared to 0.0756 l/s (2.1 log CFU/ml) and 0.108 l/s (1.9 log CFU/ml). *E. coli* O157:H7 populations in water peaked in the water/lettuce experiments at 50/45, 35/40 and 20/25 s for flow rates of 0.0756, 0.108, and 0.189 l/s, respectively, with no *E. coli* detected after 75/95, 65/100 and 30/80 s.

Significance: Bacterial removal rate from the lettuce increased significantly with water velocity, which is important to consider in future models of bacterial transfer and/or associated risk analyses.

Poster

P1-145 The Effect of Water Hardness on the Efficacy of Sodium Hypochlorite Inactivation of *Eschericha coli* O157:H7 in Water

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Introduction: Fresh produce grows in natural environments and is susceptible to microbial contamination. Sodium hypochlorite is often added to wash water by the fresh produce industry to prevent cross-contamination during postharvest washing. Many of the fresh produce processing facilities in the U.S. are located in regions with hard water. The effect of water hardness on the efficacy of sodium hypochlorite in inactivating *Escherichia coli* O157:H7 remains to be determined.

Purpose: This study examines how the hardness of water affects the efficacy of sodium hypochlorite in inactivating *E. coli* O157:H7 in water.

Methods: Water was prepared at different degrees of total hardness (0, 50, 100, 200, 500, 1000, 2000, and 5000 mg/l $CaCO_3$). Trials were performed at different levels of sodium hypochlorite (0, 0.2, 0.5, and 1 ppm). Thirty ml of chlorinated water was inoculated with 6 log CFU/ml of *E. coli* O157:H7 and allowed to mix for 3, 10, 20, or 30 seconds. After the specified exposure time, sodium thiosulfate was added to neutralize the sodium hypochlorite. The level of *E. coli* O157:H7 in the treated water was determined by plate counts.

Results: In the absence of sodium hypochlorite, no reduction in *E. coli* O157:H7 counts were seen regardless of degree of water hardness. After a 10 second exposure to 0.5 ppm sodium hypochlorite, *E. coli* O157:H7 counts were reduced by 5.3 ± 0.5 log CFU/ml in water containing 0 mg/l $CaCO_3$. In contrast, when the water contained 100, 1000, 2000, or 5000 mg/l $CaCO_3$, *E. coli* O157:H7 counts were only reduced by 1.6 ± 0.4, 0.9 ± 0.6, 0.1 ± 0.0, or 0.1 ± 0.1 log CFU/ml, respectively, after 10 seconds exposure to 0.5 ppm sodium hypochlorite.

Significance: Water hardness may need to be taken into account when determining the effective level of sodium hypochlorite needed to inactivate *E. coli* O157:H7 in wash water.

P1-146 Efficacy of Neutral Electrolyzed Water in Reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on Fresh Produce Items Using Modified Washing Procedures and Simulated Food Service Operation Conditions

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Developing Scientist Competition

Introduction: Foodborne illness incidences have been on the rise in recent years with a substantial number being linked to fresh produce contamination. A number of these outbreaks have been linked to food consumed in a food service establishment.

Purpose: The objective of this study was to determine the efficacy of Neutral Electrolyzed Water (NEW) in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on romaine lettuce and tomatoes washed in an automated salad spinner. The effects of different washing-speeds (40 and 65 rpm) and treatment times (1 to 30 min) were tested.

Methods: Whole lettuce leaves and tomatoes were spot-inoculated with 100 µl of a mixture of 5 strains of either *E. coli* O157:H7 or *S.* Typhimurium DT 104 and stored for 24h at 4°C to simulate food service operations. Washing produce in a modified salad spinner apparatus was preceded by rinsing under running NEW (155 mg/l free chlorine) whereas for lettuce an additional rinsing with NEW in spinner for 30 sec was included. Treatment with deionized water served as a control and six samples of each treatment were analyzed.

Results: Time and washing-speed had significant effects ($P < 0.05$) on the efficacy of NEW with reductions between 1.22 to 4.16 log CFU/g and 2 to 5.85 log CFU/g observed for *E. coli* O157:H7 and *S.* Typhimurium DT 104, respectively, on lettuce. Washing tomatoes with NEW also led to reductions greater than 5 log CFU/fruit for both pathogens and up to 8 log CFU/fruit for *S.* Typhimurium DT 104. Treatments with NEW completely inactivated pathogens in wash solutions (detection limit, 0.3 log CFU/ml).

Significance: Results suggest that NEW is effective in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on romaine lettuce and tomatoes and can therefore be used in food service kitchens to ensure produce safety.

P1-147 A Multi-step Screening Procedure for Selecting Surrogate Organisms for Use in Validating Fresh Produce Washing Processes

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Introduction: Chlorine-containing washes are used in post-harvest processing of fresh produce. Validation of this preventative control will be required under the Food Safety Modernization Act, but safe-to-use surrogates which mimic the pathogen of concern are unavailable.

Purpose: To develop a procedure by which surrogates can be identified for use in validating leafy greens washing processes.

Methods: A multi-step screening procedure including growth in nutrient media, chlorine susceptibility, and re-growth after chlorine exposure was developed for identifying potential surrogates for *E. coli* O157:H7. Potential strains were obtained from enrichment cultures of chlorine-washed lettuce (5-10 ppm for 30 sec), as well as enrichments of commercial probiotics and lactic acid bacterial (LAB) species. *E. coli* O157:H7 associated with leafy greens outbreaks were used as reference strains. Initial selection was based on growth at 25 and 37°C, with elimination of strains showing low OD_{600} turbidity. Lag phase time and max OD_{600}were compared using Bioscreen-C. The remaining candidates were exposed to chlorine (0-10 ppm) for 30 sec in a minimum inhibitory concentration (MIC) format. After neutralization and incubation MICs were compared to reference strains. Growth and re-growth of exposed cultures were also monitored on agar and by Bioscreen-C. Strains with higher chlorine susceptibility by three dilution levels or having atypical re-growth compared to references were eliminated. The pool was further narrowed after 16S-RNA typing and elimination of potentially unsafe strains.

Results: A pool of 80 isolates, including 60 from the chlorine-exposed lettuce, 8 probiotic enrichment isolates and 12 LAB were tested. Atypical growth compared to references resulted in elimination of ~ 15 candidates. Approximately 50 isolates were eliminated after chlorine exposure and re-growth. Several of the remaining 15 candidates have been 16S-RNA typed and will be considered for evaluation in bench-scale washing processes.

Significance: A practical screening method for identifying surrogates was developed.

P1-148 Withdrawn

P1-149 Microbial Profiles of Selected Fresh Produce from Farmers' Markets and Retail Stores in Illinois

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Introduction: Consumer demand for fresh produce and proximity of produce that can be marketed in the local markets without major quality losses are critical factors contributing to the rapid increase of farmers' markets in last decades in U.S.A. However, there has been no documented study assessing the microbial safety risk of locally-grown produce in comparison with produce from major supply chains sold in grocery stores.

Purpose: The purpose of this study is to assess and compare the microbial profiles of three fresh vegetables from farmers' markets and retail stores.

Methods: Seventy-two farm stands in 28 farmers' markets in Illinois were randomly selected for sample collection. Eleven retail stores in Illinois were chosen for comparing the microbial loads. The samples were collected from June, 2013, to October, 2013. The three vegetables included lettuces (n = 43), pepper (n = 64) and tomatoes (n = 68). APC, generic *E. coli* and coliform counts were determined using 3M™ Petri film. *Salmonella* sp. and *E. coli* O157:H7 were analyzed by FDA BAM.

Results: No *Salmonella* spp. and *E. coli* O157:H7 were detected. APC and coliform of pepper (4.61 and 1.35 mean log CFU/g) and tomato (4.18 and 1.19 mean log CFU/g) from the farmers' markets were not significantly different from those of pepper (4.32 and 1.62 mean CFU/g) and tomato (3.7 and 0.69 mean CFU/g) from the retail stores. However, APC and coliform of lettuce from the farmers' markets were significantly different from those from the grocery stores. Percent *E. coli* positive in lettuce (13.64%), pepper (7.14%), and tomato (2.17%) from the farmers' markets were little higher than those (9.52, 4.76 and 0 %) of retail markets. There were no significant differences between coliform and aerobic plate counts when analyzed by farmers' market size, location, and sampling time.

Significance: The data collected provided useful information for determining food safety issues of locally grown produce and development of food safety training materials for produce farmers.

P1-150 Microbial Safety of Fresh Herbs from Los Angeles, Orange County, and Seattle Farmers' Markets

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Introduction: Farmers' markets have been growing in popularity along the West Coast of the United States. These markets are generally held during the summer months and the produce is stored in open booths at ambient temperatures. Currently, there is a gap in knowledge regarding the microbial quality and safety of the items sold at farmers' markets in the United States.

Purpose: The purpose of this study was to assess the microbial safety and quality of fresh basil, parsley, and cilantro sold at farmers' markets in the Los Angeles, Orange County, and greater Seattle areas.

Methods: A total of 133 samples (52 basil, 41 cilantro, and 40 parsley) were collected from 13 different farmers' markets and they were tested for *Salmonella* and generic *E. coli*. The samples were tested for *Salmonella* using a method adapted from the Food and Drug Administration Bacteriological Analytical Manual, while levels of generic *E. coli* were determined using *E. coli*/Coliform Petrifilm plates.

Results: Although 15 fresh herb samples were found to be suspect for *Salmonella*, only one parsley sample confirmed positive. This gave parsley a 2.5% prevalence rate for *Salmonella*. For generic *E. coli,* 21.8% of the samples showed growth on Petrifilm plates with a range of 0.70-3.15 log CFU/g. Among the herbs tested, basil showed the highest percentage of samples with growth for generic *E. coli* (25.0%), followed by cilantro (22.0%), and then parsley (20.0%). Overall, these results suggest a moderate frequency of trace level fecal and environmental contamination, with a minor fraction of samples being highly contaminated, in fresh herbs sold at farmers' markets.

Significance: The results from this study emphasize the importance of maintaining proper storage temperatures and thoroughly washing fresh produce before consumption.

P1-151 Prevalence of *Salmonella*, *Escherichia coli* O157:H7 and *Shigella* in Selected Fresh Produce from Supermarkets, Local Markets and Farmers' Markets

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Introduction: *Salmonella, Escherichia coli* O157:H7 and *Shigella* were often responsible produce-associated foodborne illness outbreaks in the U.S. Cilantro, green onions, jalapeños and serrano peppers are often eaten raw or used in uncooked sauces and salsas in Asian and Mexican cuisines. Therefore, it is important to recognize the pathogens that affect the microbiological quality of these types of fresh produce.

Purpose: To evaluate the total microbial populations of *Salmonella*, *E. coli* O157:H7 and *Shigella* on cilantro, green onions, jalapeños and serrano peppers purchased at supermarkets, local markets, and farmer's markets.

Methods: A total of 201 cilantro, green onion, jalapeño and serrano pepper samples from seven cities were purchased and analyzed. For aerobic plate count (APC), coliform bacteria (CB) and *E. coli* detection, petrifilms were used. U.S. Food and Drug Administration Bacteriological Analytical Manual procedures were followed for *Salmonella*, *E.* coli O157:H7 and *Shigella* assays. Populations of microbial loads were calculated and analyzed by ANOVA (SAS 9.4).

Results: *E. coli* loads ranged from 1.18 to 2.42 log CFU/g in four positive samples. APC load on collected samples varied from 4.61 to 8.56 log CFU/g, and CB levels ranged from 0 to 5.68 log CFU/g, respectively. Levels of CB and APC on cilantro were significantly higher than loads on the other three types of produce ($P < 0.05$). CB levels on farmer's market produce samples were significantly higher than levels on samples from local markets and supermarkets ($P < 0.05$). No *Salmonella, E. coli* O157:H7 or *Shigella* was detected.

Significance: This study provides more information on the microbial loads of cilantro, green onions, jalapeños, and serrano peppers purchased at different venues. The results suggest that consumers should increase their vigilance when handling these items, particularly since they are often consumed raw.

P1-152 Laboratory and Industrial-scale Examination of Post-harvest Chlorine and Chlorine Dioxide Antimicrobial Applications for Whole, Fresh Gala Apples

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Poster

Introduction: Antimicrobial interventions must be validated using industry-relevant parameters.

Purpose: Chlorine and chlorine dioxide were evaluated as post-harvest antimicrobials for whole, fresh apples in laboratory and commercial settings.

Methods: In replicated laboratory studies, apples (560-850) inoculated with generic *Escherichia coli* (*E. coli)* or *E. coli* O157:H7 were randomly assigned to: inoculated untreated, chlorine or chlorine dioxide (oxidation-reduction potential {ORP} of 665mV, 750mV and 850mV), phosphoric acid (pH 3.5) or water, for 2, 3.5 and 5 minutes. Samples were enumerated on violet red bile agar and Cefixime-Tellurite Sorbitol-MacConkey agar. At commercial facility 1, 975 apples (3 replications) were randomly assigned to: inoculated untreated, or inoculated and treated in a dump tank containing water, phosphoric acid (pH 3.5), chlorine (targeted 750 and 850mV ORP), chlorine dioxide (750 and 850mV ORP) or peroxyacetic acid (80ppm). At commercial facility 2, 1,600 apples (4 replications) were examined at: 1) chlorinated dump tank with low or high organic loads 2) hyperwash containing chlorine dioxide with low or high organic loads 3) chlorinated flume system at high organic load, low organic load-750mV ORP chlorine and low organic load-850mV ORP chlorine.

Results: For laboratory studies, microbial levels after treatment with water, phosphoric acid, chlorine (665mV, 750mV, or 850mV) or chlorine dioxide were statistically similar. At facility 1, chlorine (targets of 750 and 850mV) in the dump tank achieved a significant, 0.9 log reduction in generic *E. coli* levels on apples in low organic load water. At facility 2, in the presence of low organic load, generic *E. coli* levels on apples collected after the chlorine dump tank treatment were significantly reduced (1.5 log), while in the presence of high organic loads, generic *E. coli* levels on apples collected after the dump tank were reduced 0.8 log.

Significance: Commercial experiments demonstrated larger antimicrobial reductions than laboratory experiments. Antimicrobial effectiveness in commercial facilities depends on multiple factors.

P1-153 Survival of *Listeria monocytogenes* and *Salmonella* spp. on the Epicarp of Avocados (*Persea americana* var. Hass) as Affected by Simulated Transport Conditions

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Introduction: Mexico is the major exporter of avocado to the United States. Avocados may become contaminated with pathogens at the farm or the packing facility, before shipping to the US. Avocados are transported under refrigeration to delay ripening and spoilage. If transportation conditions deviate, pathogens attached to the surface of avocados may survive or multiply.

Purpose: To determine the survival of *Listeria monocytogenes* and *Salmonella* on the surface of avocados as affected by simulated transport conditions.

Methods: Unripe non-washed avocados (n = 432) were surface inoculated (steam scar and epicarp) with a bacterial cocktail containing six rifampicin-resistant (Rf+) strains of *L. monocytogenes* or six Rf+ *Salmonella* strains (≈9 log CFU/ml). The avocados were maintained at 25°C for 1h, randomly divided into groups and stored in a chamber at 5, 12 or 25°C. At times 0, 3 and 6h, and every 24h for 10 days, two avocados from each group were sampled. Ten cm^2 surface samples from each inoculated area were excised and combined to enumerate loosely and strongly attached cells. Each experiment was conducted in triplicate.

Results: After 10 days at refrigeration (5.2 ± 0.5ºC; 83.7 ± 5.4% RH), *L. monocytogenes* counts were reduced from 4.1 ± 1.1 and 3.3 ± 0.9 to 1.0 ± 0.7 and 0.3 ± 0.7 log CFU/cm^2 for loosely and strongly attached cells, respectively, while *Salmonella* loosely and strongly attached cells were reduced from 3.8 ± 0.5 and 3.6 ± 0.8 to 1.8 ± 1.2 and 2.0 ± 0.9 log CFU/cm^2, respectively ($P < 0.05$). Bacterial counts for both pathogens were also reduced ($P < 0.05$) after 10 days at 12.3 ± 0.8ºC (85.6 ± 2.9% RH). At 24.9 ± 0.4°C (89.2 ± 5.8% RH), only *L. monocytogenes* counts were reduced ($P < 0.05$).

Significance: *L. monocytogenes* and *Salmonella* survived for 10 days on the surface of avocados under refrigeration and temperature abuse conditions. If avocados become contaminated during harvesting or processing, these pathogens will survive during the time required for avocado transportation from Mexico to the US.

P1-154 Efficacy of Peroxyacetic Acid against *Salmonella* and Native Microbiota in Float Tank Water Used during Commercial Walnut Hulling

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Introduction: During postharvest hulling, water-filled float tanks that are commonly used to separate inshell walnuts from rocks, sticks, and soil are a potential vehicle for microbial cross-contamination.

Purpose: To evaluate the efficacy of a peroxyacetic acid (PAA)-based sanitizer against *Salmonella* and native microbiota in float tank water collected from a commercial walnut huller.

Methods: PAA (25, 50, and 80 ppm) was assessed against a five-strain cocktail of rifampicin-resistant *Salmonella* and, separately, the native microbiota in both fresh well and used float tank water (collected after 1h of hulling). Duplicate individual samples from each of three separate trials (n = 6 per treatment) were neutralized with Dey/Engley broth after 30, 60, 90, and 120 s of exposure to PAA. Appropriate dilutions were plated onto tryptic soy (TSA) and bismuth sulfite agars, both with rifampicin (*Salmonella*), TSA with cycloheximide (aerobic plate count (APC)), CHROMagar ECC (coliforms), and Rose Bengal chloramphenicol agar (fungi) and incubated at 37°C for 24h or 22°C for 96h for bacteria or fungi, respectively. Total dried solids and consumption of peroxyacetic acid were determined.

Results: Populations of *Salmonella*, APC, coliforms, and fungi were 5.9, 0.6, <–1.0, and <–1.0 log CFU/ml, respectively, in the well water and 5.9, 6.6, 5.9, and 6.0 log CFU/ml, respectively, in the used float tank water. Reductions of >5.0 log CFU/ml were observed for *Salmonella* within 120 or 30 s of exposure to 25 or 50 and 80 ppm PAA, respectively, in both well and used waters. Populations of APC, coliforms, and fungi were reduced 2.3, >5.0, and 2.6 log CFU/ml, respectively, after 30 s of exposure to ≥25 ppm PAA in used float tank water. Total dry solids were 37 to 126 times higher in the used float tank water. PAA (80 ppm) was consumed at a rate of 0 and 1.3 ppm/min in well and used float tank water, respectively.

Significance: Use of a PAA-based sanitizer may reduce the potential for microbial cross-contamination during walnut hulling by controlling microbial loads in float tank water.

P1-155 Preliminary Survey of the Occurence of Foodborne Human Pathogens in Pecan Production Fields

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Introduction: Recent outbreaks of foodborne illnesses associated with tree nut consumption have increased the attention to food safety issues related to tree nuts. Currently, there is limited information about the natural occurrence and potential sources or contributing factors leading to tree nut contamination by human pathogens.

Purpose: The aim of this study was to assess the natural occurrence of human pathogens, Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella*, in native pecan production fields.

Methods: Samples of soil, cattle feces, pecans, and wild animal fecal swabs were collected from different sites of three pecan production fields at preharvest (two months before harvesting) and during harvest. Swabs from harvesting equipment surfaces were also collected during harvest. Isolation and detection of STEC and *Salmonella* were performed by enrichment, selective enrichment/plating, and multiplex PCRs. Molecular typing of isolates was conducted by pulsed-field gel electrophoresis (PFGE).

Results: At preharvest, *Salmonella*. was detected from 22%, 17%, and 18% of soil, cattle feces and wild animal swabs, respectively. However, none was detected from samples collected during harvest except 1 of 16 equipment surface swabs. Similarly, STEC were detected at preharvest from 22%, 100%, and 45% of soil, cattle feces and wild animal swabs, respectively. During harvest, these numbers were 22, 50%, and 50%, respectively, while 2 of 16 equipment surface swabs were positive for STEC. None of the harvested and bagged pecans were tested positive for either pathogen; however, STEC were isolated from 5 samples of broken pecans that had been excluded from bagging. Identical PFGE patterns were observed between STEC isolates from soil and cattle feces, and broken pecan and one of the harvesting equipment surface swabs.

Significance: This study showed the presence of human pathogens in pecan production fields and harvesting equipment, highlighting the need for the development of mitigation strategies starting from the production field to control potential human pathogen contamination of pecans.

P1-156 Prediction of *Listeria monocytogenes* Presence in a Frozen-vegetable Processing Plant Using Traditional Microbial Indicators and *Listeria* spp

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Developing Scientist Competition

Introduction: In food-processing environment, the prediction of *Listeria monocytogenes* presence through the use of microbial indicators is not always effective, because most of them do not share ecological characteristics with this pathogen. Particularly, in ready-to-eat products such as frozen vegetables, there isn't information regarding relationship between microbial indicators (including *Listeria* spp.) with *L. monocytogenes.*

Purpose: The main goal of the present study was to determine the relationship between microbial indicators, *Listeria* spp. and *L. monocytogenes* in a frozen-vegetable processing plant.

Methods: During one year, samples from contact-surfaces, non-contact surfaces and vegetables (broccoli, carrots, cauliflower, cob, pea, and champignon) were collected and analyzed by conventional bacteriological methods. In all the samples, the presence of *Listeria* spp. and *L. monocytogenes* was determined. Also, aerobic plate count (APC), total coliforms (TC) and lactic acid bacteria (LAB) were quantified exclusively in contact-surfaces samples. The relationship between *Listeria* spp. and *L. monocytogenes* was determined by Pearson test, and the correlation of microbial indicators with *L. monocytogenes* was calculated by logistic regression.

Results: A total of 574 samples were analyzed. The incidence of *Listeria* spp. and *L. monocytogenes* in vegetables, contact-surfaces and non-contact surfaces was 10.04 and 7.53, 9.29 and 5.58, 28.78 and 27.27 %, respectively. In all samples, a positive correlation ($P < 0.0001$) was found between *Listeria* spp. and *L. monocytogenes*. In samples from contact-surfaces, the estimated median of APC, TC and BAL was 4.25, 2.62 and 2.92 log CFU/100 cm^2, respectively; only APC and TC showed a significant correlation with the pathogen presence ($P < 0.05$).

Poster

Significance: The present study suggests that *Listeria* spp. could be an effective microbial predictor of the presence of *L. monocytogenes* in vegetables and surfaces in this industry. Additionally, APC and TC count worked as good indicators of the pathogen on contact-surfaces.

P1-157 Emergence of Carbapenem-resistant Enterobacteraceae: From Healthcare to Harvest

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Introduction: The emerging carbapenemase group classified as Amber Class B Metallo-β-Lactamases (MBL's) has been an increasing concern worldwide. Recent reports of *Klebsiella pneumonia* producing IMP, VIM, and NDM types have heightened interest over additional transmissible carbapenem- resistance in *Enterobacteriaceae*. Carbapenem-resistant *Enterobacteriaceae* (CRE) is primarily associated with the healthcare field but now is becoming a food safety issue.

Purpose: The objective of this study was to isolate and identify Metallo-β-Lactamases producing *Staphylococcus aureus* from porcine and produce samples.

Methods: The 127 samples tested were comprised of 50 porcine and 77 produce samples. The strains were isolated and identified according to the procedure of the Bacteriological Analytical Manual with some modification to identify the presence of *Staphylococcus aureus.* DNA was collected using PrepSEQ Rapid Spin Sample Preparation Kit in accordance with manufacturer's instructions and then Polymerase Chain Reaction (PCR) was performed.

Results: Results demonstrated that 5.2 % of samples collected produced NDM, 23.4% produced VIM, 18.2% produced SPM-1, and 15.6% produced IMP.

Significance: In conclusion the data is in support of the spread of class B MBL's into foods and is deserving of concern and immediate actions.

P1-158 Effects of Physical Variables on *Salmonella* Transfer from Produce to Stainless Steel

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Developing Scientist Competition

Introduction: Prior work has suggested that bacterial transfer from produce to contact surfaces during slicing is affected by surface roughness, relative contact speed, distance, and normal force. However, mathematical models of these relationships have not been well developed, as prior studies typically tested overall transfer results, but did not elucidate single-variable effects.

Purpose: The objective was to quantify the effect of four physical variables on *Salmonella* transfer to stainless steel during sliding contact with potatoes used as the model product.

Methods: Peeled potatoes were cut into 3-cm cubes, spot-inoculated with *Salmonella* Typhimurium LT2 (~6 log CFU/cm^2), and then pulled (using a controlled speed-force machine) across a 304 stainless steel plate with variations in surface roughness (brushed vs. mirror finish), sliding speed (2, 5, 8 mm/s), total contact distance (20, 30, 180 cm), and additional mass placed on the product (30, 60, 90 g) to obtain different normal forces. After contact, Kimwipe® samples collected from the potato/stainless steel contact path were appropriately diluted and plated on modified trypticase soy agar to quantify *Salmonella*. Bacterial populations along the contact path were analyzed via a repeated measures statistical analysis.

Results: Greater transfer ($P < 0.05$) was seen to mirror-finished stainless steel. Overall, normal force did not significantly affect transfer, except at long contact distances; however, contact speed and distance impacted cumulative transfer ($P < 0.05$) for certain cases. For example, greater cumulative transfer ($P < 0.05$) occurred over 30 cm of contact at 5 mm/s than at 2 mm/s (420,000 vs. 190,000 CFU total).

Significance: Quantifying the effects of individual physical variables is critical to the future development of bacterial transfer models and the refabricating/redesigning of fresh-cut processing equipment and related produce-handling operations to minimize cross-contamination.

P1-159 Effect of Sanitizer and in-package Atmosphere on *Listeria monocytogenes* Growth in Diced Yellow Onions

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Introduction: In 2012, a large recall was issued for *Listeria monocytogenes*-contaminated commercially diced onions, which has shaken consumer confidence.

Purpose: This study aimed to assess the effect of several sanitizers and in-package atmospheres on the fate of *L. monocytogenes* in diced onions stored at 7°C.

Methods: Spanish jumbo yellow onions (*Allium cepa*) were diced using an Urschel Model H-A dicer, dip-inoculated with a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) at 5.9 log CFU/g and air-dried for 8 min. The onions were then washed for 2 min in 60 l of 4°C water containing either no sanitizer, 80 ppm free chlorine (XY-12, Ecolab) adjusted to pH 6.5 with citric acid, 80 ppm peroxyacetic acid (PAA) (Tsunami 100, Ecolab), or 2 ppm ClO_2 (CDG Environmental). After centrifugal drying, 100-g samples of diced onion were packaged in 11 x 11 cm polylactic acid (PLA) pouches (EVLON EV-HS1, BI-AX International Inc.) flushed with 99% O_2 or air. Periodically during 14 days of storage at 7°C, one package of diced onions per treatment was added to PBS buffer, homogenized by stomaching, appropriately diluted, and then plated on Modified Oxford Agar to enumerate *Listeria*. Findings from triplicate experiments were analyzed by the Tukey-Kramer HSD test using JMP 10.

Results: Initial *Listeria* reductions were significantly ($P < 0.05$) greater for PAA (1.9 log CFU/g) as compared to water, chlorine, and ClO_2 (0.9 - 1.0 log CFU/g). After 14 days of storage at 7°C, the total increase in numbers of *Listeria* was significantly ($P < 0.05$) lower in 99% O_2 (0.6 log CFU/g) as compared to air (1.6 log CFU/g) with no significant ($P > 0.05$) difference observed between sanitizers.

Significance: Based on these findings, packaging diced onions in PLA bags with a high oxygen atmosphere can reduce the extent of *L. monocytogenes* growth during extended refrigeration.

P1-160 Rapid Detection and Quantification of *Salmonella enterica* in Peanut Butter by a rRNA Detection System

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Introduction: Methods for rapid and reliable detection and quantification of microorganisms and pathogens in food, beverages and water are receiving increasing attention. The sandwich hybridization method used in the rRNA Detection System is a suitable alternative for such analysis. This test method is independent of the influence of sample matrices, and is able to distinguish between live and dead cells. Furthermore, the detection of non-culturable microbes is possible. This test performed in less than 3 hours (in addition to the prep time), offers a significant time savings compared to cultivation-based assays.

Purpose: Rapid detection and quantification of pathogens like *Salmonella enterica* for economical and safe production of food and beverages.

Methods: *Salmonella enterica* subsp. enterica (ATTC 13311) was detected by the use of HybriScan***D*** *Salmonella* Test. The method is based on molecular genetic identification allowing detection of a group of microorganisms as well as specific species. No PCR is required because the method is quantitative without cell counting (using standards) and uses standard laboratory equipment. The signal read-out is triggered optically by an enzymatically generated color change. Peanut Butter sample matrix (9 different brand codes with 12 different code dates) was examined. Peanut butter samples inoculated with *Salmonella enterica* were tested along with a control. All samples and the negative controls were tested and verified for *Salmonella* according to EN ISO 6579:2002.

Results: Out of 107 *Salmonella enterica* inoculated peanut butter samples, 106 were identified as clearly contaminated with *Salmonella* by the use of HybriScan***D*** *Salmonella* assay. The result of 1 sample was considered questionable. All negative controls gave negative results in the HybriScan***D*** *Salmonella* assay. 1-5 CFU of *Salmonella* spp. in 25 g Peanut Butter can be positively identified by HybriScan***D*** *Salmonella* assay after 24 hours enrichment time. Results have shown the possibility to shorten the enrichment time of pre-and main culture for the HybriScan***D*** *Salmonella* Test from 42 hours to a total of 24 hours for Peanut Butter matrix.

Significance: HybriScan rRNS Detection System is an economical, high throughput, 96-well microplate format system. The test is performed in less than 3 hours (in addition to the prep time) and offers a significant time savings compared to cultivation-based assays.

P1-161 Determination of the Dry Heat-resistance of *Salmonella* spp. and *Eschericha coli* O157:H7 Dried on to Stainless Steel Surfaces in Low Loisture Food Production Environments

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Introduction: It is known that the dry heat resistance of members of the pathogenic species *Salmonella enterica* and some other vegetative organisms is greatly increased at low water activity, potentially allowing resistant populations to survive thermal decontamination processes. This affect is known to vary between different low moisture foods. However, very little published data is currently available concerning the heat resistance of such organisms under low moisture conditions in dry food processing environments.

Purpose: To determine the heat resistance characteristics of *Salmonella* Typhimurium ATCC 14028 and a desiccation-resistant strain of *Salmonella* Enteritidis across a 20°C temperature range, providing data demonstrating the effect of dry heat on *Salmonella* and *Escherichia coli* O157:H7 ATCC 43888 on stainless steel surfaces. To facilitate the design of thermal treatments which may potentially allow decontamination of sections of process lines, equipment and food contact surfaces.

Methods: *Salmonella* and *Escherichia coli* O157:H7 strains were dried onto the surface of stainless steel discs and exposed to dry heat on the surface of an aluminium block in an oil bath at 95, 100, 105, 110 and 115°C (for *Salmonella* spp.) or 75, 80, 85, 90 and 95°C (for *Escherichia coli*). In all cases, survivor curves were used to calculate D and z values for the organisms tested.

Results: Analysis of the death kinetics of *Salmonella* Typhimurium ATCC 14028 and *S.* Enteritidis on stainless steel discs exposed to dry heat has given z-values for these strains of 24.4 and 28.7C° respectively. Dry heat D-values on stainless steel at 105°C were found to be 70.5 and 51.4 minutes respectively. Initial testing of *Escherichia coli* O157:H7 and *Cronobacter sakazakii* on stainless steel gave D-values of <3 minutes and 9.9 minutes respectively at 95°C.

Significance: When compared with previously published D-values determined for *S.* Typhimurium ATCC 14028 in low a_w nuts & seeds (102.4-242.1 minutes, Campden BRI R&D report no. 244, 2013), D-values have been found to be lower on stainless steel, but still considerably higher than might be expected for *Salmonella* spp. heated under moist conditions (>6 log reductions after 2 minutes at 70°C). Initial testing of *C. sakazakii* and *E. coli* O157:H7 on stainless steel suggests that these organisms may not be as heat resistant as *Salmonella* under low a_w conditions.

P1-162 Impact of Inoculation Procedures on Thermal Resistance of *Salmonella* in Wheat Flour and Associated Repeatability of Results

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Developing Scientist Competition

Introduction: Investigation of *Salmonella* inactivation typically involves artificial inoculation of a food matrix. However, most such studies focus exclusively on the effects of the treatment variable (e.g., product composition), neglecting to consider the influence of inoculation procedures.

Purpose: The objective was to quantify the impact of five different inoculation methods on thermal resistance of *Salmonella* Enteritidis PT30 in wheat flour, and subsequent repeatability in a two-laboratory comparison study.

Methods: Batches of wheat flour (100 g) were inoculated with *Salmonella* Enteritidis PT30 by five different methods: (A) high-concentration, low-liquid volume (HCLV) broth culture, (B) HCLV suspended lawn culture, (C) pelleted and resuspended lawn culture, (D) direct contact with a lawn culture, and (E) fomite transfer of a lawn culture. After inoculation, samples were equilibrated (~5 d) to ~0.45 a_w in a controlled-humidity chamber, subjected to isothermal (80°C) inactivation trials in aluminum test cells in a water bath (11 durations in triplicate), immediately cooled in ice water, serially diluted, and plated on modified trypticase soy agar with yeast extract. D-values were computed from the resulting log CFU/g data by linear regression.

Results: Post-equilibration and post-come-up *Salmonella* populations ranged from 8.7 to 6.3 and 7.7 to 3.7 log CFU/g, respectively. Method A yielded the largest population decline during equilibration (~3 log) and come-up (~2.5 log) and also the highest D-value (504.9 s), compared to the other methods ($P < 0.05$). The MSU-generated D-values for methods B, C, and D were clustered (250.9, 285.9, and 226.7 s, respectively), but statistically different ($P < 0.05$).

Significance: Based on these findings, careful consideration should be given to the inoculation method, which can significantly impact thermal resistance of *Salmonella* in low-moisture foods, and the inherent uncertainty, which can significantly affect utility of resulting models.

P1-163 Improving Safety of Wheat Milled Products through Processing

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Introduction: Wheat is subject to microbial contamination; consequently enteric pathogens, such as *Salmonella* spp. and *Escherichia coli*, may be among its microflora creating a food safety risk in milled products. Since the microbial flora of wheat is the primary contamination source, it is important to mitigate the risk prior to milling.

Purpose: The purpose of this study was to evaluate the effectiveness of organic acids and saline solutions as antimicrobial treatments against *Salmonella* spp. and *E. coli* O157:H7, during wheat tempering.

Methods: To inoculate the wheat, cocktails were prepared using either five serotypes of *S. enterica* or five kanamycin-resistant strains of *E. coli* O157:H7. To prepare the inoculum, each strain/serotype was grown independently at 37°C for 24h, and mixed in equal proportions. Wheat was then inoculated to achieve a 5.0 log CFU/g, followed by a resting time (24h). Besides water, solutions containing acid (acetic or lactic - 2.5% or 5%) and salt (NaCl - 1%) were used for tempering (24h) the wheat. Samples were then plated before and after tempering on Tryptic Soy Agar (TSA) with an overlay of Xylose-Lysine-Tergitol 4 agar for *S. enterica*, and TSA+kanamycin (50µl/g) for *E. coli* and incubated (37°C for 24h). The log reduction in the pathogenic populations was calculated and differences in treatments compared using ANOVA.

Results: The initial load of pathogens was reduced significantly by all treatments when compared to the control which was tempered with water ($P < 0.05$). The best results for *S. enterica* and *E. coli* O157:H7 were achieved using lactic acid 5%+NaCl 1% solution, which resulted in 2.0 and 1.8 log reduction in CFU/g, respectively.

Significance: Implementation of organic acids and salt in tempering water prior to milling could benefit the milling industry and consumers by preventing or reducing the risk of microbial contamination in milled products.

P1-164 Decline of *Salmonella* on Artificially Contaminated Dry Pet Food

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Introduction: Due to several outbreaks and large-scale product recalls, *Salmonella* has emerged as a priority pathogen in dry pet food and treats. However, little data is available to quantify the risk posed by this class of products to both pets and their owners. Specifically, the kinetics of *Salmonella* survival on this complex food matrix composed of fats, protein, and grains has not been characterized.

Purpose: This study sought to measure the short- and long-term kinetics of survival or decline of *Salmonella* in dry pet food under storage conditions (low water activity and ambient temperature) commonly encountered during production, at retail, and at households.

Methods: A *Salmonella enterica* cocktail of 12 strains isolated from pet food and treats was used to inoculate 25-g batches of commercial dry pet food at approximately 9.5 log CFU/sample. After air drying for 8 hours, inoculated samples were stored in airtight containers at room temperature (21-23°C). At each sampling time, *Salmonella* was enumerated in three inoculated and one negative sample by stomaching in 0.1% peptone water, preparing serial dilutions, and plating on generic (BHI) and selective (XLD and BSA) media using a spiral plater. Water activity was also measured. Resulting data points were fitted using log-linear and non-linear decline models using the R software.

Results: Results at 83 days highlight a fast and approximately log-linear initial decline (up to 54 days), followed by a phase of lower decline rate. A Weibull model (Log CFU ~ Log CFU_0 - (1/delta)t^p) provided a satisfactory overall fit (delta: mean 6.91, SE 2.76 log CFU/days; p: mean 0.48 Log CFU/days, SE 0.07). Water activity remained constant at 0.48-0.50.

Significance: This study provides a complete decline model that can feed into quantitative risk assessments.

P1-165 Influence of Sucrose Content on the Survival of *Salmonella* in Low-water Activity Whey Protein Model Systems at 70°C

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Developing Scientist Competition

Introduction: *Salmonella* spp. contamination has been and continues to be problematic within the food industry. Numerous *Salmonella* spp. outbreaks have been associated with low-water activity foods. Many products recalled for *Salmonella* contamination contain sugar. However, little is known about the effect of sugar on survival of *Salmonella* in foods at low-water activity.

Purpose: The purpose of this study was to determine if sucrose content of low-water activity protein powder affects the survival of various *Salmonella* species independently of water activity.

Methods: A four strain cocktail, *S.* Typhimurium, *S.* Tennessee, *S.* Agona, and *S.* Montevideo, was dried and added to whey protein powder that contained concentrations of 0, 5, and 20% (w/w) sucrose. Samples were equilibrated to the targeted water activity level before inoculation. Inoculated samples were treated at 70°C for 48 hours with samples tested for survivors at various times. *Salmonella* were recovered from treated samples using supplemented tryptic soy agar. Log values of survivors at different sugar contents and times were compared using ANOVA.

Results: Sugar content had no effect on survival of Salmonella in the lowest water activity samples (a_w 0.18). These treatments produced a decline in the *Salmonella* population from 10^9 CFU/g to log reductions of 2.9 for 0%, 3.5 for 5%, and 2.3 for 20% sucrose samples over 48 hours. A protective effect of sucrose was observed at a_w 0.52 after 4 hours of heat treatment. This treatment achieved an average log reduction of 3.2 for samples containing 0 and 5% sucrose and a 2.3 log reduction for samples containing 20% sucrose.

Significance: The data suggests that effects of sucrose content in dry food on the survival of *Salmonella* during heat treatment are determined primarily by water activity. This observation will assist in the development of predictive models.

P1-166 Dry Talc Inoculum Preparation Procedure with *Salmonella* and the Surrogate (*Enterococcus faecium*) for Challenge Studies in Low-moisture Foods

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Introduction: The food industry is preparing for the proposed Preventive Controls rule, recently published under the Food Safety Modernization Act, addressing preventive control measures for the biological hazards associated with low-moisture foods. To accomplish this task, it is crucial to have a validated surrogate for process validation, and validated inoculation procedure.

Purpose: The objective of this study was to obtain dry inocula of *Salmonella* Tennessee and *Enterococcus faecium* and compare their stability over time in terms of survival and thermal resistance.

Methods: Two methods of cell growth were used: (1) cells harvested from lawns on tryptic soy agar (TSA-cells), and (2) cells from tryptic soy broth (TSB-cells), for dry inoculum preparation. Concentrated cultures of each organism were inoculated onto talc powder, followed by incubation at

35°C for 24h, and held for additional 24h at room temperature (RT ~25°C) for complete dryness before sieving. The recovery of dry inoculum was monitored on tryptic soy agar. Heat resistance at 85°C of the dry inoculum on talc inoculated in a model peanut paste (50% fat and a_w= 0.6) was determined for up to 30 days.

Results: The dry inoculum on talc for both cell-growth methods was stable in terms of survival and thermal resistance over 30 days. In general, the dry inoculum on talc resulted in a higher cell concentration when TSA grown-cells were used, compared to TSB. However, the TSB grown cells had a significantly (P < 0.05) greater heat resistance than TSA grown-cells (e.g., *E. faecium* TSB-cells $D_{85°C}$ = 3.43 min versus 2.68 for TSA-cells). The surrogate had consistently greater heat resistance than *Salmonella* Tennessee (e.g., TSB-cells $D_{85°C}$ = 1.05 min), regardless of what cell-type was used for dry inoculum preparation.

Significance: A dry inoculum of the surrogate for *Salmonella* appears to be more relevant and easier to use for process validation in low-moisture foods.

P1-167 Thermal Resistance of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104 and *Enterococcus faecium* in Peanut Paste Formulations at Two Levels of Water Activity (0.3 and 0.6) and Fat Concentrations (47 and 56%) at 75°C

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Introduction: The increased heat resistance of *Salmonella* in low-moisture foods is affected by many factors, including food matrix used during heating (e.g., a_w and fat concentration). Heat resistance observed in an aqueous system is not applicable to a low-moisture product.

Purpose: The objective of this study was to determine and compare the heat resistance of *Salmonella* Tennessee and Typhimurium DT104 with that of *E. faecium*, a potential surrogate for thermal inactivation of *Salmonella* in model peanut pastes (two levels of a_w (0.3 and 0.6) and two concentrations of fat (47 and 56%)).

Methods: Four peanut pastes containing 47% or 56% fat at 0.3 or 0.6 a_w, were inoculated with dry inoculum on talc (~1x10^6 CFU/g) and screened for thermal resistance at 75°C. A thermal death test method using copper plates to compress the peanut paste samples at ≤ 1 mm thickness was used. Counts of survivor cells were used to generate the inactivation curves using the Geeraerd and Van Impe Inactivation Model Fitting Tool in the xla program; for statistical analysis Minitab Release 14 software was used.

Results: There was no significant (P > 0.05) difference between the heat resistance of *Salmonella* Tennessee and *Salmonella* Typhimurium in tested conditions (e.g., the minimum calculated time for 2 decimal reductions (2D) was 25.4 min for Tennessee and 24.2 for Typhimurium at 47% fat and 0.3 a_w). Maximum log-reduction (4.0) of *Salmonella* Typhimurium occurred at 56% fat at both a_w, implying that fat level might be a more critical factor than a_w in heat inactivation of this organism under these conditions. The inactivation rates of *E. faecium* were consistently lower (e.g., 74.7 min for 2D at 47% fat and 0.3 a_w).

Significance: Understanding how fat and a_w can affect the heat resistance of *Salmonella* in food will allow a better control of this pathogen; *E. faecium* might be an appropriate and conservative surrogate for thermal process validation.

P1-168 Effect of Hot Air Roasting Process on the Survival of *Salmonella* spp. and *Listeria monocytogenes* during Sunflower Seeds Processing

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed low moisture foods highlight the need for food industries to validate their thermal process as a part of their food safety system.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of *Salmonella* spp. and *Listeria monocytogenes* during sunflower seeds processing.

Methods: Sunflower seeds samples were individually inoculated with cocktails of *Salmonella* spp. and *L. monocytogenes* to achieve a target level of 10^8 CFU/g. Following inoculation, samples were brined and roasted at oven settings of 225°F or 275°F. Roasting times were between 5 to 45 minutes. The study constituted 3 replicates. Samples inoculated with *Salmonella* spp. were enumerated using Tryptic Soy Agar (TSA) pour plates overlaid with Xylose-lysine-desoxycholate agar. Samples inoculated with *Listeria monocytogenes* were enumerated using TSA pour plates overlaid with Modified Oxford Agar. Water activity analysis was performed using AquaLab Dew Point Water Activity Meter 4TE. Log-transformed data were fitted to a multiple regression model using least squares regression. 95% prediction intervals were generated to predict the minimum thermal processing parameters required to achieve a 4-log reduction in *Salmonella* spp. and *L. monocytogenes.*

Results: Modeling of sunflower seed data indicated that to meet a minimum 4-log reduction in *Salmonella* spp. and *L. monocytogenes,* thermal processing parameters of 275°F (hot air temperature) for 20 minutes were needed. A water activity of 0.620 and product temperature of 180°F were found to be the corresponding food safety verification parameters. The adjusted R-square values ranged from 0.91-0.99.

Significance: The study findings indicate that the hot air roasting process achieves acceptable lethality of vegetative pathogens. The data generated in this study provides scientific basis for the facility's food safety plan.

P1-169 Thermal Resistance of *Salmonella* during Brewing Related to Length of Storage of Inoculated Green Tea Leaves

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Introduction: *Salmonella enterica* has been implicated in outbreaks linked to brewed tea. *Salmonella enterica* is well known for its increased thermal resistance under low moisture conditions, although, it remains unclear if the increased resistance is a physiological response or due to inefficient heat transfer through the low moisture environment.

Purpose: The purpose of this work was to determine if length of storage of tea leaves inoculated with *Salmonella* resulted in increased thermal resistance during subsequent brewing.

Methods: Sterilized dried gunpowder green tea leaves (1 g) were inoculated with *Salmonella enterica* serovar Anatum (0.1 ml, approximately 11 log CFU/ml) , then dried to a water activity of approximately 0.2. The thermal resistance of *Salmonella* was determined after inoculation of tea, after storage (25°C; ~20% RH) of tea for 24h, and after 28 days storage. Nine ml deionized water was added to 1 g inoculated tea leaves in Whirl-pak bags, then immediately submerged in a circulating water bath (55°C). At various time points, three bags were removed, and *Salmonella* enumerated. Each experiment was conducted three times resulting in 9 samples for each time point.

Results: The D_{55}-values for *Salmonella* in tea using freshly inoculated tea leaves, inoculated tea leaves after 24h storage, and after 28 day storage were 2.78 ± 0.12, 3.04 ± 0.07, 2.78 ± 0.56 min, respectively. Although there was no statistical difference in the D-values ($P > 0.05$), the variability (standard deviation) of the associated D-value increased with storage.

Significance: No change in the thermal resistance of *Salmonella* in tea was observed related to the length of storage of the inoculated tea leaves. However, an increase in the variability did occur and could reflect attachment/detachment to tea leaves.

P1-170 Impact of Refrigerated Storage on Thermal Inactivation of *Enterococcus faecium* NRRL B-2354 and *Salmonella* Enteritidis PT 30 ATCC BAA-1045 on Inoculated Almonds

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Introduction: *Enterococcus faecium* NRRL B-2354 is a recognized surrogate for *Salmonella* Enteritidis phage type 30 for thermal processes applied to almonds. Almond Board of California guidelines allow for surrogate-inoculated almonds to be stored at 3.3 to 4.4°C, but the impact of refrigerated storage on the thermal tolerance of *E. faecium* is unknown.

Purpose: To assess the survival and thermal resistance of *E. faecium* and *Salmonella* Enteritidis on inoculated almonds during refrigerated storage.

Methods: *E. faecium* and *Salmonella* Enteritidis were harvested from agar lawns and then inoculated onto almonds; air-dried inoculated almonds were stored at 4 ± 2°C for up to 12 weeks. Inoculated almonds ($n = 6$, from two trials) were sampled at bi-weekly intervals and were treated at 138 ± 2°C for 15 min in either a gravity feed or forced air (*E. faecium* only) oven on aluminum mesh trays. The hot almonds were immediately transferred to cold tryptic soy broth and shaken, stomached, and serially diluted in Butterfields phosphate buffer before plating onto tryptic soy agar (*Enterococcus* and *Salmonella*) and bismuth sulfite agar (*Salmonella*).

Results: Populations of *E. faecium* and *Salmonella* Enteritidis on almonds did not significantly decrease throughout 12 weeks of refrigerated storage. At all time points, reductions of *Salmonella* Enteritidis were 2.7 ± 0.1 log CFU/g upon heat exposure in the gravity feed oven. In contrast, reductions of *E. faecium* increased steadily from 1.5 log CFU/g at time 0 to 2.3 log CFU/g at week 12, and differences were significant between weeks 0 and 1 and week 12. Reductions of *E. faecium* were significantly ($P < 0.05$) higher in the forced air oven than in the gravity feed oven.

Significance: Further research is necessary to support long-term storage of *E. faecium*-inoculated almonds for validation studies.

P1-171 Determining Validity of *Enterococcus Faecium* as Surrogate for *Salmonella* under Stagnant Dry Heating of Peanuts

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Developing Scientist Competition

Introduction: *Enterococcus faecium* ATCC 8459 is used as a surrogate for *Salmonella* in thermal processing of almond. It is not validated as for use in peanut processing.

Purpose: Our objective was to evaluate *E. faecium* as a surrogate for *Salmonella* during peanut thermal processing.

Methods: Peanut kernels (unblanched, medium runner) were inoculated to ca. 7-8 log CFU/g, after drying to original % moisture and water activity (a_w), with nalidixic acid resistant strains of *S.* Enteritidis phage type (SEPT) 30, *S.* Seftenberg, *S*. Tennessee or *E. faecium* (ATCC 8459). Kernels (50 g) were exposed to dry heat at 120 ± 1°C (20, 30, 40 min), 130 ± 1°C (10, 20, 30 min) or 140 ± 1°C (10, 20, 30 min) (n = 6). Populations were enumerated on selective and non-selective media supplemented with nalidixic acid (50 µg/ml). a_w and % moisture were measured before inoculation, after inoculation, after drying and after each time-temperature combination.

Results: To be considered a good surrogate, log reductions of *E. faecium* must be significantly equal to or less than those of *Salmonella*, indicating it is more heat resistant. Under the conditions tested, log reductions of *E. faecium* were significantly higher ($P < 0.05$) than SEPT30 (120°C: 20 min; 130°C: 20, 30 min; 140°C: 10 min), *S.* Seftenberg (130°C: 20, 30 min; 140°C: 10 min) and *S.* Tennessee (130°C: 30 min). A log reduction exceeding 5 log CFU/g was only seen for *S.* Tennessee after 30 min at 140°C. Calculated D- and z-values were highest for SEPT30; D-values: 15.2 min at 120°C, 11.3 min at 130°C, and 7.9 min at 140°C and z-value 70.4°C. Decreases of up to 4.85 % moisture and 0.25 a_w occurred following treatment.

Significance: *Enterococcus faecium* does not appear to be a valid surrogate for *Salmonella* during thermal processing of peanuts under stagnant dry air conditions; under forced dry air conditions, results may differ.

P1-172 Effect of Inoculation Method on the Survival of *Salmonella* Tennessee on Whole Black Peppercorns

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Developing Scientist Competition

Introduction: Increased incidence of foodborne illness associated with *Salmonella* contaminated spices has highlighted an urgent need for validated inactivation methods. In order to develop reliable processes, more understanding about the role of the inoculation strategy on the recoverability of *Salmonella* strains from the spice surface is needed.

Purpose: To compare the effects of inoculation method and physiological state of cells (planktonic or biofilm) on the recoverability and stability of *Salmonella enterica* ser. Tennessee on whole black peppercorn.

Methods: Whole black peppercorns were inoculated with *S*. Tennessee cells using a wet inoculation method where planktonic cells grown for 24h in TSB were applied directly to peppercorns. Alternately, biofilms were formed on peppercorns over a 24h period of static incubation in TSB. Peppercorns were dried to $a_w = 0.3$, and held for 28 days. Peppercorns (n = 3) were sampled after drying (time 0), and after 1, 14, 21, and 28 days of storage. Cells were enumerated by serial dilution and plated onto XLT-4 and TSA.

Results: In general, recovery of *Salmonella* was high after 28d storage, with averages of 4.31 log CFU/g and 5.15 log CFU/g recovered for planktonic and biofilm cells, respectively. Plate counts from peppercorns inoculated using the two methods were comparable throughout the study, with the exception of the 14 day sample, in which the biofilm counts (5.89 log CFU/g) were statistically greater ($P = 0.0186$) than the planktonic counts (4.90 log CFU/g). The log reduction from initial inoculation levels after 28 days was significantly larger ($P = 0.0176$) for planktonic cells (1.73 log reduction) than the biofilm cells (0.03 log reduction).

Significance: The inoculation method influenced the recoverability of *Salmonella* from whole peppercorns after drying. Identifying a standardized strategy for inoculation of whole peppercorns is an imperative step toward developing a standardized protocol for validation testing of commercial spice processing methods.

P1-173 The Influence of Fat Content on the Survival of *Salmonella* in a Low-water Activity Model Food System at 70°C

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Developing Scientist Competition

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

Purpose: The purpose of this study is to evaluate the role of fat in the survival of *Salmonella* in a whey protein isolate model food system at 70°C and at constant water activities of 0.18 and 0.57.

Methods: Peanut oil was homogenized with whey protein isolate to achieve 5, 10 and 20% (w/w) fat concentration and was equilibrated to water activity levels of 0.57 and 0.18. A dried cocktail including *S.* Typhimurium*, S.* Tennessee*, S.* Agona*,* and *S.* Montevideo was inoculated and treated at 70°C for 24 hours (0.18 a_w) and 16 hours (0.57 a_w). Survivors were recovered at various times using supplemented Tryptic Soy Agar. Log CFU/g of surviving *Salmonella* in 0, 5, 10 and 20% peanut oil at each time point were compared using one-way and two-way ANOVA.

Results: Overall survival was not affected by fat content after 16 hours at 0.57 a_w and 24 hours at 0.18 a_w (P = 0.841, df =3, α=0.05). Survival of *Salmonella* differed with water activity (P = 0.001). In addition there were no differences in survival at individual time points (P > 0.05). The average log reduction of *Salmonella* was 4.93 at 0.57 a_w after 16 hours and 2.67 at 0.18 a_w after 24 hours.

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

P1-174 Effect of Almond Product Structure on X-ray Inactivation Kinetics of *Salmonella*

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Introduction: Microbial contamination of low-moisture foods is a difficult food safety challenge, reflected in several nationwide *Salmonella* outbreaks and/or recalls involving different types of nuts and various other dry ingredients. Therefore, effective processing interventions that minimally impact end-product quality are needed to reduce the risk of *Salmonella*.

Purpose: The purpose of this study was to assess the efficacy of X-ray irradiation for inactivation of *Salmonella* on three structurally different almond products - whole kernels, almond meal, and almond butter under the same environmental conditions.

Methods: Almond kernels were inoculated with *Salmonella* Enteritidis PT30 and processed into meal and butter using a blender, after which the three structurally different products were conditioned in a controlled-humidity chamber. Each sample was bagged inside the chamber and irradiated at two dose levels using an x-ray food irradiator at 70 kV (Rayfresh, Ann Arbor, MI). Thereafter, the irradiated samples were stomached, serially diluted, and plated on modified trypticase soy agar to enumerate survivors. Data from three replicates were plotted with the *D*-values determined by linear regression.

Results: After 4 weeks of storage, almond kernel, meal, and butter yielded *Salmonella* populations of 7.22, 7.54, and 7.61 CFU/g which were less than 1 log below the initial inoculation level. The water activity at the time of irradiation was 0.333, 0.339, and 0.315, and the radiation *D*-value was 0.430 (R^2=0.92), 0.363 (R^2=0.97), and 0.318 (R^2=0.98) kGy for almond kernels, meal, and butter, respectively.

Significance: X-ray irradiation was an effective means to inactivate *Salmonella* Enteritidis PT30 in/on various almond products. However, the process needs to be calibrated for different types of products, given that the efficacy can be affected by structural differences, even at identical composition and water activity.

P1-175 Effect of Product Structure on Thermal Resistance of *Salmonella* Enteritidis PT30 on Whole Almonds, in Almond Meal and in Almond Butter

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Developing Scientist Competition

Introduction: Low-moisture products, such as almonds, can be contaminated with *Salmonella*in the production environment. Subsequent valued-added processes change product structure, but the impact on pathogen thermal resistance has not been reported.

Purpose: The objective was to quantify the effect of product structure on thermal resistance of *Salmonella* Enteritidis PT30 inoculated onto whole almonds subsequently ground into almond meal and almond butter.

Methods: Almonds were inoculated with *Salmonella* Enteritidis PT30 (~10^8 CFU/g) and equilibrated to ~0.4 a_w. After equilibration, almonds (100 g) were ground in a food processor (45 s) to produce an almond meal sized between U.S. standard sieves #20 and 80. Almond butter was produced by further milling almonds (200 g) for 15 min, with dry ice added every 2 min to control product temperature (<40°C). All products were re-equilibrated to ~0.4 a_w. The inoculated almonds were individually vacuum-packed in thin layer plastic bags, and meal and butter samples (thickness < 1 mm) were packed in aluminum test cells. Samples were heated in an isothermal water bath (~80°C), with almonds pulled every 10 min for 1.25 h, and meal and butter pulled every 15 min for 2.5 h; all were cooled immediately in an ice bath, diluted in peptone water, and plated on modified TSA to enumerate survivors.

Results: Initial *Salmonella* populations and sample water activities were not significantly different (P > 0.05) after grinding and milling. However, $D_{80°C}$ values, determined by linear regression of the *Salmonella* survivor curves, were greater (P< 0.05) in almond meal (60.7 min) and almond butter (66.0 min) than on the whole almonds (19.1 min).

Significance: Changing the product structure, given equivalent a_w and composition, significantly impacted *Salmonella* thermal resistance. Therefore, it is extremely important to use product-specific inactivation parameters when validating pasteurization processes.

Poster

P1-176 Inactivation of *Salmonella* in Low-moisture Products at Relatively High Temperatures Using Radiofrequency-assisted Heat Treatments (RFHT)

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Developing Scientist Competition

Introduction: *Salmonella* outbreaks in low-moisture foods create significant need for effective pasteurization processes, but conventional thermal treatments are challenged by low thermal diffusivity of dry products. Radiofrequency (RF) energy has reportedly reduced heating time and reduced *Salmonella* in low-moisture products such as spices without significant quality damage.

Purpose: The goal was to assess inactivation of *Salmonella* Tennessee K4643 (ST) and Enteritidis PT30 (SPT30) in low-moisture products using RFHT.

Methods: Product (100 g) was inoculated (≈9 log CFU/ml) with ST or SPT30 using a lawn culture pellet re-suspended in peptone water (3 ml). Samples were equilibrated in a controlled-humidity chamber to ~0.45 a_w, then isothermally (80°C) treated in aluminum cells in an oil bath (5 durations, 6 replicates), immediately cooled in ice water, serially diluted, and plated on XLT4 agar. D-values were obtained by linear regression of the survival data (log CFU/g) and used to design RFHT. Samples (~20 g) were heated to ~80°C in a 0.5 kW, 27 MHz RF unit and immediately transferred to an oil bath for different holding times (duplicates), then cooled, diluted, and plated as previously explained.

Results: D-values for SPT30 (5.3 ± 0.56 min, 0.93 R^2) and ST (5.7 ± 0.61 min, 0.91 R^2) in wheat flour were not significantly different ($P > 0.05$). RFHT yielded average log reductions immediately after RF treatment, after 6 min, and after 12 min holding time, respectively, of 2.02 ± 0.31, 3.09 ± 0.30, and 3.27 ± 0.39, for ST, and 3.06 ± 0.19, 4.18 ± 0.14, and 3.60 ± 0.54 for SPT30. Only the SPT30 6 min holding time lethality was significantly higher ($P < 0.05$) than obtained with test cells. RFHT inactivation was not statistically different ($P > 0.05$) between strains.

Significance: RFHT is a promising technology for *Salmonella* inactivation in low-moisture products, and inactivation kinetics are essential to future process design and validation.

P1-177 Efficacy of Dry and Moist Heat on the Inactivation of *Salmonella* in a Low-moisture Powder Residue Attached to Stainless Steel Surfaces

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Poster

Introduction: Validating sanitation protocols for low-moisture food processing equipment (dry cleaning) is challenging. Although heat can be used to reduce *Salmonella*, the relative effect of humidity and temperature on inactivation rates in low-moisture products and environments has not been well quantified.

Purpose: The purpose of this study was to quantify the effect of temperature and humidity on the inactivation of *Salmonella* and a common non-pathogenic surrogate (*Enterococcus faecium*) in a low-moisture powder on stainless steel.

Methods: A soy-based protein powder was inoculated (~10^9 CFU/g) with a 3-strain cocktail of *Salmonella* (Enteritidis PT30, Tennessee, and Enteritidis PT4) or *E. faecium* (NRRL B-2354), adhered in a thin-layer (~0.5 mm) to stainless steel coupons, equilibrated to ~0.175 a_w, then heated in a pilot-scale moist-air impingement oven (T_{db} = 93, 121, or 149°C; T_{dp} = ~33, 60, or 71°C; v_{air} = 1.2 m/s; 3 reps). Surviving *Enterococcus* and *Salmonella* were enumerated by plating recovered samples on deMan, Rogosa, and Sharpe agar or modified trypticase soy agar, respectively. Protein residue temperatures were measured using surface thermocouples on the coupons, and used to calculate D-values for both organisms.

Results: For T_{db} = 121°C, and T_{dp} of ~33, 60, or 71°C, the D-values for *Salmonella* and *E. faecium* were 34, 21, 9 and 20, 18, 10 min, respectively. For all but the highest humidity (71°C) the *E. faecium* inactivation rate was higher ($P < 0.05$) than for *Salmonella*. At T_{dp} of ~33°C, increasing T_{db} from 121 to 149°C reduced ($P < 0.05$) the *Salmonella* D-value from 34 to 1.5 minutes (R^2= 0.98, 0.98).

Significance: Air temperature and humidity both significantly affected inactivation rates for *Salmonella* and *E. faecium*; however, utilization of the surrogate for process validation would require comparison to *Salmonella* resistance at the specific process conditions.

P1-178 Survival of *Salmonella* on Dried Fruits and in Aqueous Dried Fruit Homogenates as Affected by Temperature

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Introduction: Dried fruits have not been considered as likely vehicles of foodborne pathogens. However, documented presence of pathogens in dried fruits and recent evidence showing that *Salmonella* can survive on dried fruits for at least 3 months have raised interest in learning more about survival during long-term storage.

Purpose: The purpose of this study was to (1) determine the ability of *Salmonella* to survive on four dried fruits stored at 4 and 25°C and (2) determine if *Salmonella* can grow in aqueous homogenates of dried fruits.

Methods: Dried cranberries (a_w 0.47), date paste (a_w 0.69), raisins (a_w 0.46), and strawberries (a_w 0.21) were mist- or dry-inoculated with a five-serotype mixture of *Salmonella*. Inoculated fruits stored at 4 and 25°C were analyzed for *Salmonella* at 1-week to 2-month intervals up to 8 months. Survival and growth of *Salmonella* in 10 and 50% aqueous homogenates of dried fruits were monitored over a 12-week period.

Results: *Salmonella* survived on cranberries, date paste, raisins, and strawberries stored at 25°C for 21, 84, 21, and 42 days, respectively. The pathogen survived at 4°C on cranberries, date paste, and raisins for at least 242 days (8 months) and on strawberries for at least 182 days (6 months). Compared to cells grown in broth, cells grown on an agar medium survived longer in date paste. *Salmonella* did not grow in 10 and 50% fruit homogenates stored at 4°C but grew in 10% date paste and raisin homogenates at 25°C.

Significance: Results suggest the need to subject dried fruits that may be contaminated with *Salmonella* to a lethal process before eating out-of-hand or using as ingredients in ready-to-eat foods. The ability of *Salmonella* to grow in aqueous homogenates of date paste and raisins emphasizes the importance of minimizing contact of these and perhaps other dried fruits with high-moisture environments during handling and storage.

P1-179 Effect of Rapid Desiccation on Thermal Resistance of *Salmonella* in Wheat Flour

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Developing Scientist Competition

Introduction: *Salmonella* is able to survive in low moisture environments, and has been shown to become more resistant to heat as the water activity (a_w) of the product decreases. However, it is unknown how rapidly the resistance changes if the product water activity is rapidly altered, as can occur in certain processes.

Purpose: The purpose of this study was to evaluate the effect of rapid desiccation on the thermal resistance of *Salmonella*.

Methods: Wheat flour was inoculated with *Salmonella* Enteritidis PT30 (~8.0 log CFU/g), then divided into three treatment groups. Groups A and B were equilibrated over ~4 d in controlled-humidity chambers to 0.6 and 0.3 a_w, respectively. Group C was equilibrated to 0.6 a_w, then rapidly dried to 0.3 a_w (< 4 min), using desiccated room temperature air in a small fluidized bed drying system. Samples (~1 g) then immediately (within ~1 min) were isothermally treated (80°C) in aluminum test cells for varying durations, immediately cooled in ice water, serially diluted, and plated on modified trypticase soy agar with yeast extract for enumeration of survivors. D-values were calculated and compared via ANOVA.

Results: The mean D-values for groups A, B, and C were 1.33, 7.32, and 5.73 min, respectively. The rapidly desiccated group (C) and the group initially equilibrated to 0.3 water activity (B) were not significantly different ($P > 0.05$), but both were significantly greater than for the group initially equilibrated to 0.6 water activity ($P< 0.05$).

Significance: *Salmonella* in the rapidly desiccated flour (0.3 a_w) was as thermally resistant as that which previously had been equilibrated to 0.3 a_w. These results suggest that the observed enhanced thermal resistance of *Salmonella* at lower a_w is a state function that requires negligible adaption time.

P1-180 *Salmonella* Biofilm Formation on Peppercorns and Polystyrene

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Developing Scientist Competition

Introduction: Several recent outbreaks traced to *Salmonella* contaminated spices have highlighted an emerging vehicle. *Salmonella* may persist on spices and on food contact surfaces within a biofilm matrix.

Purpose: The purpose of this study was to compare the biofilm-forming capabilities of multiple strains of *Salmonella* on a whole spice (black peppercorns) and polystyrene.

Methods: Biofilm-forming capacity of 14 *Salmonella* strains on polystyrene was examined using a multi-well plate reader. Biofilm development after 48h of growth in TSB was determined by staining the wells with crystal violet, washing plates to remove loose cells and measuring their optical densities. Biofilms of seven strains were formed on whole black peppercorns by statically incubating a single layer of whole peppercorns in *Salmonella*-inoculated TSB for 48h at 37°C. Peppercorns were washed twice to remove planktonic cells, dried to a water activity of 0.42, serially diluted and enumerated by plating onto selective media.

Results: Of the 14 strains tested on polystyrene, two strains were predicted to have poor biofilm-forming capacity (OD: 0.16-0.21) and twelve strains were predicted to have medium biofilm-forming capacity (OD: 0.21-0.42). Seven strains belonging to the medium biofilm-forming category were screened for formation on whole black peppercorns. Differences in biofilm formation on the two surfaces were observed for several strains. *Salmonella* Orion had medium biofilm-forming capacity on polystyrene but was not recovered from washed peppercorns. *Salmonella* svs. Tennessee and Johannesburg had very high recovery from peppercorns (7.43 ± 0.02 and 7.42 ± 0.02 log CFU/g, respectively) but had greatly varying optical densities (0.164 and 0.266, respectively) as determined by the crystal violet assay.

Significance: *Salmonella* biofilm formation on peppercorns and polystyrene differs by serovar and surface.

P1-181 Thermal Resistance of *Salmonella* in Low-moisture Foods

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Introduction: In the last five years, there have been nine outbreaks associated with low-moisture foods including nuts, spices, pet food, and extruded snack foods, involving *Salmonella*, which led to over 1,300 cases of foodborne illness and nine deaths. If contaminated at any point from harvest through packaging, the final ready-to-eat foods could lead to foodborne illness, especially if processing steps are unable to inactivate food pathogens.

Purpose: This project will develop simple predictive model systems for microbial heat resistance for pathogenic strains of *Salmonella* by determining the thermal resistances of *Salmonella* serovars in moist, intermediate, and low-water activity environments.

Methods: *Salmonella* (n = 10) associated with low-moisture foods were grown on tryptic soy agar with yeast extract, harvested via physical removal, and inoculated into buffered peptone water (BPW), corn syrup, peanut butter and flour. Samples (80µL) were individually heated at a prescribed rate (5°C/min) to 55-90°C in a differential scanning calorimeter. Surviving *Salmonella* was enumerated via plate count. D- and z-values were calculated by plotting log (log N_0/N) *versus* temperature. The inverse slope of the line is the z-value. The D-value at a desired temperature is determined by the y-intercept at that temperature

Results: As water activity decreased there was a significant increase in D-values for all *Salmonella* tested ($P < 0.05$). While all *Salmonella* investigated were more resistant in corn syrup ($D_{70°C}$ = 2.15-156 min) than BPW ($D_{70°C}$ = 0.01-0.18 min), the thermal resistance of the serovars did not follow the same resistance trend. In BPW *S.* Tennessee and *S.* Agona exhibited the greatest thermal resistance, with a $D_{70°C}$ = 0.18 min. In corn syrup *S.* Typhimurium and *S.* Anatum were the most thermally resistant, $D_{70°C}$ = 52.6 and 156 min, respectively.

Significance: As *Salmonella* serovars exhibit different thermal death kinetics depending on matrix, development of these predictive models will be essential for establishing preventive controls for these pathogens in low-moisture foods.

P1-182 Apple, Orange, and Strawberry Juices Treated with Pulsed Light (PL). Effect on Some Inoculated Microorganisms and Native Flora during Refrigerated Storage

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Introduction: PL has been shown to inactivate inoculated pathogenic and spoilage microorganisms in a variety of food products. However its effect on product shelf life is controversial.

Purpose: This research was aimed to investigate native flora as well as some inoculated microorganisms (*Escherichia coli* ATCC 35218; *Listeria innocua* ATCC 33090, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE162) evolution in the following fresh squeezed juices: apple (pH: 3.4; 12.7°Brix), orange (pH: 4.3; 10.4°Brix), and strawberry (pH: 3.6; 9.8°Brix), after being exposed to PL and during refrigerated storage.

Methods: For each PL treatment (Xenon lamp; 3 pulses/s; 60 s; 71.6 J/cm^2), 0.1 ml of inoculum (~5x10^8 CFU/ml) was added to the juice (4.9 ml) contained in a Petri dish refrigerated with ice flakes (T < 20°C). PL treated samples were collected in 10 ml caramel jars and kept under refrigerated conditions (5 ± 1°C) for 12 days. At periodic time intervals they were analyzed for survivors. Non inoculated juices were used for the shelf-life study. Experiences were performed in quintuplicate.

Results: PL treatment produced 1.4 - 2.6-log reductions in apple juice depending on the strain; while 0.3 - 0.8-log reductions were obtained in orange and strawberry juices. A significant decrease of 5.0 - 8.0 log cycles was determined at the end of storage for *S.* Enteritidis and *L. innocua* in all PL treated juices. Non growth was observed for *E. coli* and *S. cerevisiae*, except in apple juice, where the yeast was able to recover starting from day 9 of storage . PL reduced up to 1.4 and 1.5 log cycles molds and yeasts and aerobic mesophilic counts, respectively; however it did not prevent their recovery along storage.

Significance: PL was effective providing microbiological stability regarding relevant microorganisms along refrigerated storage but it did not improve juice shelf life, suggesting the requirement of its combination with other hurdles in order to reach the desired storage stability.

P1-183 Study of the Inactivation of *Saccharomyces cerevisiae* in Apple Juice by High Intensity Pulsed Light (PL) and Ultrasound (US): Assessment of the Physiological Status by Flow Cytometry (FC) and Transmission Electron Microscopy (TEM)

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Introduction: A hurdle strategy to improve PL effectiveness could be its combination with US.

Purpose: This study was aimed to analyze the damage on *S. cerevisiae* in apple juice provoked by a US+PL treatment through the study of the physiological characteristics by FC and the ultrastructure by TEM.

Methods: Commercial (pH 3.5; 12.5°Brix) and natural squeezed apple juices were inoculated (pH: 3.4; 11.8°Brix) with *S. cerevisiae* KE162 (~1 x 10^6 CFU/ml). Ultrasonic treatments (20 kHz, 600 w; 95.2 µm; 20, 30 or 44°C; 10 and 30 min) were applied. US treated or not juice samples were exposed to PL (Xenon lamp; 3 pulses/s; 60 s; 71.6 J/cm^2; $T_{initial}$: 2; 30; 44°C) reaching different final temperatures due to the heat build-up during PL treatment (T_{final}: 13; 42; 56°C). After treatments, cells were labelled with fluorescein diacetate (FDA) and propidium iodide (PI) for monitoring esterase activity and membrane integrity, respectively. After conventional fixation, cells were examined by TEM using a JEOL transmission electronic microscope.

Results: Yeast log reduction was up to 1.8-2.8 for single US, 2.0-3.8 for single PL, and 5.8-6.4 for US+PL in natural squeezed or commercial apple juice, respectively. When single US was applied, FC revealed that population gradually migrated from PI^--FDA^+ to PI^+-FDA^- quadrants, indicating rupture of membranes and progressive loss of esterase activity(EA) (10 min-US: 2.9-11.6 % EA; 30 min-US: EA non detected). When US+PL treatments were applied, EA was not detected and membrane permeability significantly increased, depending on juice type and temperature. In TEM, sonicated cells showed puncturing of walls with leakage of content and damage at subcellular level. When PL was applied, inner content did not leach out of the cells but appeared coagulated and the lumen looked coarse.

Significance: FC and TEM analysis helped to better understand inactivation mechanism by US+PL combined treatment.

P1-184 Evaluation of a Rapid Approach for the Detection and Direct Identification of Spoilage Microorganisms in Fruit Juices

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Introduction: Spoilage microorganisms of the genera *Alicyclobacillus, Lactobacillus, Candida, Leuconostoc, Saccharomyces* and *Zygosaccharomyces* are of major concern to the fruit beverage industry. They have different growth characteristics and often require multiple growth media and temperatures for their detection and isolation. A single medium based automated detection system along with Matrix Assisted Laser Desorption Time-of-Flight Mass Spectrometry (MALDI-TOF MS) based identification can simplify and reduce the total time to quality assessment of fruit beverages.

Purpose: Evaluation of a single broth based medium in an automated system for the detection of spoilage microorganisms from fruit juices followed by direct identification using MALDI-TOF MS.

Methods: Twenty-five strains of genus *Alicyclobacillus*, *Lactobacillus, Leuconostoc, Candida, Saccharomyces* and *Zygosaccharomyces* were separately inoculated (10-25 CFU) in 15 ml orange and apple juice samples in triplicate. The inoculated samples were mixed in 20 ml BacT/ALERT *i*LYM medium bottles and incubated at 32±0.5°C in an automated CO_2 sensor based detection system. Samples (2 ml) from positive bottles were centrifuged, washed with sterile water and suspended into 50 µl ethanol, which were directly identified using a MALDI-TOF MS system. Similarly, pure cultures of all the strains were individually detected and identified.

Results: All 25 bacterial and yeast pure cultures were correctly identified using the MALDI-TOF MS system. All 75 inoculated samples each of apple and orange juices were reported positive within 2-3 days using an automated detector. The yeast and lactic acid bacteria positive samples were correctly identified at genus or species level but identification of the *Alicyclobacillus* positive samples required additional processing to complete the identification.

Significance: Rapid detection and identification of spoilage microorganisms from fruit beverages could be achieved using a combination of automated detection and MALDI-TOF MS identification, which could significantly reduce the product hold time during quality assessment and allow for earlier intervention and correction if required.

P1-185 Search for a Natural Intervention against *Listeria monocytogenes* in Wheatgrass Juice

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Introduction: Concern has risen over the microbiological safety of a locally produced wheatgrass juice product, and the most effective way to demonstrate a 5-log reduction of pathogens in this product without changing innate taste and color properties. High pressure processing (HPP) has also been shown to control microbial growth while maintaining the bioactive compounds and color of grapefruit and blueberry juices.

Purpose: The purpose of this study was to determine if HPP is effective means for achieving a 5-log reduction of *Listeria monocytogenes* in wheatgrass juice, and observe any physical changes from the processing.

Methods: Wheatgrass juice was harvested from 7-10 day old plants and inoculated with a 3-strain *L. monocytogenes* inoculum to achieve a ~7 log CFU/ml inoculation. All treatments and controls were packaged (5.0 ml) in duplicate into separate sterile Type 402 polyester pouches and heat sealed with minimal headspace. HPP treatments were applied at 586 MPa for 15 and 30 seconds. No HPP treatments were applied to the inoculation and non-inoculated controls. All samples were diluted and plated on Modified Oxford Agar (MOX) for 48 hours at 35°C. Sample enrichment was performed

with Universal pre-enrichment broth (UPB), incubated at 35°C for 48 hours and plated onto MOX agar. Color, texture, and mouth feel was determined on non-inoculated samples. Experiment was replicated three times.

Results: Following both HPP treatments no *L. monocytogenes* was observed at a detection level of 3 CFU/ml, but enrichments showed presence in 15 second treatment ($P < 0.01$). No notable differences in odor, texture or mouth feel were identified through sensory and organoleptic analysis when compared to untreated samples.

Significance: HPP at 586 MPa was effective at reducing *Listeria monocytogenes* by 7 log CFU/ml at both 30 and 15 seconds in wheatgrass juice. Further analysis should be conducted to determine if HPP processing affects textural or flavor aspects of wheatgrass juice.

P1-186 Selection of Autochthonous *Saccharomyces* Yeasts from Queretaro Vineyards Based on Safety and Enological Qualities

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Developing Scientist Competition

Introduction: The main concern about safety on wine production lays on chemical hazards such as sulfites and mycotoxins produced by microorganisms, issues widely handled by strains selection. Queretaro State in Mexico, recently holds an emerging wine industry with little information about the autochthonous microorganisms involved (among these: yeasts) and their effect in quality, tipicity and safety of the products. Thus arises the necessity to study the local yeasts' characteristics, regarding safety (sulfite production) and local needs like high sugar/ethanol yield.

Purpose: To study and select autochthonous *Saccharomyces* yeasts from Queretaro vineyards, based on their enological and safety qualities.

Methods: *Saccharomyces* strains were isolated from local grapes submitted to spontaneous fermentation and differentiated from non-*Saccharomyces* by culture in a selective media (Lysine). Afterwards they were selected based on their resistance to ethanol (12 %), sulphitation (200 ppm) and *killer* effect, performed on a turbidimetric analyzer (Bioscreen). Also fermentative and enological performances (including sulfites production) were evaluated in microvinification assays within two grape varieties. Strains selected were identified by the amplification and sequence of D1/D2 domain.

Results: From 198 isolated yeasts, 52 belonged to *Saccharomyces* spp., 8 were selected based on the resistance tests and identified by sequencing as belonging to *S. cerevisiae* and *S. paradoxus*. In the microvinification assays, strains produced dry wines with pH, volatile and titratable acidity ranging within the typical values for red wines. Moreover with statistical significance (Tukey $P \leq 0.05$), the lower values regarding SO_2 production were obtained by the strains SR19 and SR26 (below 38 mg/l), N5 (autochthonous) and K1 (control) obtained the highest sugar/ethanol yields (≈16.5g glucose/°ethanol), alcoholic degrees (≈15 %), and fermentative efficiency (≈0.91).

Significance: This study revealed the existence of autochthonous yeasts from Queretaro suitable for selection as starter cultures to improve the safety, quality and tipicity of the wines produced.

P1-187 Development of a Real-time PCR Assay for the Detection of the Spoilage Yeast *Zygosaccharomyces bailii* in Wine and Fruit Juices

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Introduction: *Zygosaccharomyces bailii* is a yeast responsible for unwanted fermentation of residual sugars in food and beverages products. Its ability to survive in environments otherwise stressful for other microorganisms makes it considered by microbiologists and quality managers as a real danger in various production environments, particularly during winemaking process.

Purpose: In order to provide a tool for controlling *Z. bailii* in beverages, a specific real-time PCR assay with associated DNA extraction method was developed allowing to recover DNA from complex matrices like wines.

Methods: This method was validated on a comprehensive panel of inclusivity and exclusivity strains naturally present in wine matrices. The performance of the DNA extraction was tested on various wine matrices from European and US origins as well as on fruit juice matrices to assess the efficiency of method to deal with potential PCR inhibitors. Eighty beverage samples corresponding to 15 different matrices (wine or fruit juices) were spiked using 2 different strains of *Z. bailii* to a theoretical level comprised between 1 and 1.8 x 10^7cell/ml.

Results: Twelve *Z. bailii* strains from different origins were assayed and were adequately detected. Conversely, 46 strains representing 26 species of yeast, mold or bacteria that can be found in wine did not yield a specific signal, thereby demonstrating the selectivity of the method. A standard calibration curve was generated demonstrating PCR efficiency of 145%, thereby establishing a good correlation between Cq values and CFU/ml of the 80 beverage samples. Finally, the assay was determined to detect as little as 1 CFU/ml.

Significance: This level of sensitivity can help food manufacturers and enologists in taking appropriate decisions for the management of wine and fruit beverages to avoid fermentation after bottling that may lead to explosion of bottles or bag-in-box.

P1-188 New Chromogenic Media for Rapid Detection of *Clostridium perfringens*

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Introduction: CP *ChromoSelect* Agar is a selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rod-shaped bacteria. It is widespread in the environment and also found in the digestive systems of humans, and domestic and feral animals. Perfringens poisoning, usually from ingesting undercooked food, especially meat, is one of the most commonly reported foodborne illnesses.

Purpose: Early detection of *Clostridium* in food and water is, therefore, important to control outbreaks.

Methods: To facilitate detection, the new chromogenic medium, the CP *ChromoSelect* Agar, for enumeration and differentiation of *Clostridium* sp., in particular *Clostridium perfringens*, is used in aqueous samples.

Three media types (mCP,TSCF and the new CP Agar) were evaluated for recovery of *C. perfringens* in different surface water samples. Using a membrane filtration technique on 139 water samples, 131 samples (94.2%) were found positive for *C. perfringens* in at least one of the culture media. Green colored colonies on the new Agar (cf. Figure 2) were counted as presumptive *C. perfringens* isolates.

Results: Out of 483 green colonies on the new Agar, 96.3% (465 strains, indole negative) were identified as *C. perfringens*, 15 strains (3.1%) were indole positive and were identified as *C. sordelli, C. bifermentans* or *C. tetani.* Only 3 strains (0.6 %) gave false positive results and were identified *as C. fallax, C. botulinum,* and *C. tertium*. Variance analysis of the data showed statistically no significant differences in the counts obtained between media

Poster

used in this work. In general, the identification of typical and atypical colonies isolated from all media demonstrated that the new Agar was the most useful medium for *C. perfringens* recovery in water samples. The new Agar avoids the disadvantages of m-CP agar, such as, the presence of ammonia that prevents subculturing the *C. perfringens* colonies, the too-selective nature of m-CP agar, and the evanescence of the red color of colonies after the addition of ammonia, which makes further confirmation impossible.

Significance: This chromogenic media is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*.

P1-189 Detection of Viable *Escherichia coli* in Environmental Water Using a Combined Propidium Monoazide Staining-real-time PCR

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Introduction: *Escherichia coli*, as a principal fecal indicator bacterium, is used to monitor water quality world-wide. Real-time PCR (qPCR) is a promising way to achieve a rapid and sensitive detection of *E. coli* in water samples. The ability to detect only viable *E. coli* cells specifically, provides a more accurate reflection of water quality and safety.

Purpose: The objective of this study was to test a new self-designed primer set targeting the *ycj*M gene of *E. coli* in a propidium monoazide (PMA)-qPCR assay, and to investigate its specificity and efficiency in detecting only viable *E. coli* in water.

Methods: Specificity of the *ycj*M primer set was checked using eight different *E. coli* strains, including *E. coli* ATCC 25922, and six other strains from environmental sources, as well as *S. dysenteriae*, *S. flexneri* and *S. sonnei*. A freshly grown culture of *E. coli* DR23 (deer isolate) was spiked into lake water and serially diluted to 10-10^7 CFU/ml. *E. coli* counts were determined by the U.S. Environmental Protection Agency (EPA) Method 1603 and samples were treated with PMA, followed by DNA isolation and amplification by SYBR® Green q-PCR targeting the *ycj*M gene primers.

Results: With the use of the *ycj*M primer set, all eight *E. coli* spp. and none of the *Shigella* spp. tested were detected by the PMA-qPCR. As low as 10^3 CFU/ml of viable *E. coli* DR23 in lake water could be detected by this method.

Significance: Compared with the EPA standard culture-based method, PMA-qPCR targeting the *ycj*M gene demonstrates a very specific and efficient alternative way to detect only viable *E. coli* in environmental waters.

P2-01 Prevalence of Pathogens and Indicators in Foods Ordered from Online Vendors in the United States

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Introduction: The widespread availability of the Internet has fostered a new business sector: online sales of perishable foods. Little information is available on the microbial safety of such foods.

Purpose: This project analyzed the microbial quality and safety of foods ordered online.

Methods: Three hundred forty-one perishable meat and seafood products were ordered from more than 80 online vendors, and delivered using common carriers including FedEx and UPS. The sampling strategy used cost of order, number of items available, and exclusion of low risk (e.g., canned) foods to maximize the number and variety of products analyzed. Data on food temperature on arrival was recorded. Foods were enumerated for the presence of indicator organisms including total plate count, coliforms and generic *E. coli*, and tested for the presence of pathogens including *Salmonella*, pathogenic *E. coli*, *Clostridium perfringens*, and *Bacillus cereus*. Seafood samples were tested for *Vibrio parahaemolyticus*, and Ready-to-Eat (RTE) samples were tested for *Listeria monocytogenes* and *Staphylococcus aureus*. Testing protocols were based on US Food and Drug Administration (FDA) Bacteriological and Analytical Manual (BAM) techniques.

Results: A total of 196 meat, 34 poultry, and 111 seafood samples were tested. Of these, 18.7% were positive for generic *E. coli*, 9.9% for pathogenic *E. coli*, 10.2% for *Salmonella*, 50.3% for *B. cereus*, 38.7% for *V. parahaemolyticus* and 18.2% for *C. perfringens*. More than 35% of the time foods were received at temperatures >4°C. A majority of *E. coli, Salmonella* and *V. parahaemolyticus* positive samples were received at temperatures >4°C. Amongst the 21 RTE samples tested, *L. monocytogenes* was detected in 4 samples. None of the samples tested positive for *S. aureus*.

Significance: This study characterizes the microbial risks associated with perishable foods ordered online. Foods are often received at temperatures >4°C and pathogen risk may be increased.

P2-02 Survival of Foodborne Pathogens in Commercial Ready-to-Bake Raw Cookie Dough in Two Different Storage Conditions

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Introduction: Since the multistate outbreak of *Escherichia coli* O157:H7 in 2009 associated with commercial ready-to-bake cookie dough, cookie dough is considered as a novel vehicle for transmission of Shiga toxin-producing *E. coli*. Additionally, use of raw eggs, a common source of *Salmonella*, as an ingredient increases the food safety risk associated with cookie dough.

Purpose: The purpose of this study was to examine the survival rate of two foodborne pathogens, *Salmonella* serovar Enteritidis and *E. coli* O157:H7 in commercial raw cookie dough during its storage.

Methods: Cookie dough samples were inoculated with 6.3 log CFU/g and 6.0 log CFU/g of *Salmonella* Enteritidis and *E. coli*O157:H7, respectively, and stored for 8 weeks at 4°C or -18°C. Samples (20 g) were taken immediately after inoculation, and then every 2 to 4 days for plate counting.

Results: During the 8 week-storage, 2.42 log reduction and 2.35 log reduction were observed at 4°C and -18°C, respectively, for *Salmonella* Enteritidis. For *E. coli*O157:H7, 2.23 log reduction and 1.99 log reduction were observed at 4°C and -18°C, respectively. This study indicates, even with a low moisture and high sugar content, pathogens can survive in cookie dough products stored under refrigeration or freezing, the conditions recommended by manufacturers, for more than 2 month.

Significance: The results suggest the prevention of pathogen contamination of cookie dough by good manufacturing practices and effective monitoring system would be the best way to prevent foodborne illnesses associated with cookie dough products. Also, it is strongly advised for consumers not to consume raw cookie dough as pathogens, if present in cookie dough, would not be easily eliminated during storage.

P2-03 Microbial Contamination Isolated from Consumer Kitchens

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Introduction: It has been suggested that food prepared in the home may be responsible for a significant amount of foodborne illness, however little is known regarding the role that the domestic kitchen plays as a potential reservoir for foodborne pathogens. While a number of studies have isolated *Listeria* and *Staphylococcus aureus* from the domestic environment there is a need to better understand the role that the domestic kitchen plays as a reservoir for a range of foodborne pathogens.

Purpose: To characterize microbial contamination and foodborne pathogens found in consumer kitchens in order to better understand the role that the domestic environment serves as a potential reservoir for infectious organisms.

Methods: Environmental samples were collected from the homes of 100 consumers in Philadelphia, PA. A total of 559 samples were collected from various surfaces in the kitchen, including the sink (n = 100), counter (n = 100), refrigerator shelf (n = 102), refrigerator drawer (n = 61), refrigerator door handle (n = 100), and a used dishcloth or sponge (n = 96). Each sample was tested for coliforms, fecal coliforms, *E. coli*, *Staphylococcus aureus*, *Salmonella*, *Campylobacter* and *Listeria* according to methods described by the Food and Drug Administration (FDA).

Results: *Staphylococcus aureus* was isolated from 13.1% of all 559 samples, most often from kitchen counter tops (17.0%), refrigerator door handles (16.0%), and kitchen sinks (14.0%). *Listeria* spp., including *L. monocytogenes* and *L. innocua,* were present on 2.3% of all samples and 9.8% of refrigerator drawers. *Escherichia coli* was isolated from 6% of kitchen sinks and 2.1% of kitchen sponges/dishcloths. *Campylobacter jejuni* was isolated from 3 households (3%), one time each from a sink, counter and dishcloth. *Salmonella* was not isolated from any kitchens in this study.

Significance: These results indicate that the domestic kitchen may serve as a reservoir for infectious organisms including *Staphylococcus aureus*, *E. coli*, *C. jejuni* and *Listeria monocytogenes*.

P2-04 Microbiological Safety of Salad Dressing Challenged with Foodborne Pathogens

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Introduction: Acidified foods (pH ≤ 4.6, a_w ≥ 0.85) traditionally have been considered safe against foodborne pathogens. However, several recent outbreaks caused by acid-resistant foodborne pathogens in some acid foods have been reported. FDA requests scientific support for cold fill and hold products through challenge study to demonstrate a 5 log reduction of pathogens (Draft Guidance for Industry: Acidified Foods).

Purpose: The objective of this study was to determine the inactivation rate of *Listeria monocytogenes, Salmonella* spp. and *Escherichia coli* O157:H7 in five formulations of salad dressing when stored at 73-77°F for 5 days.

Methods: Products were inoculated separately with 4 strain composite of each acid-adapted pathogen to achieve 10^{6-7}CFU/g. After inoculation, product was sub-sampled and stored in sterile conical tubes (30 g each) at 73-77°F. Three replicate samples were enumerated for each pathogen daily for 5 days. Once the counts were below the detection limit (10 CFU/g), samples were qualitatively analyzed for presence of each pathogen.

Results: The average initial counts of *L. monocytogenes, Salmonella* spp. and *E. coli* O157:H7 in 5 formulations ranged 5.23-5.38, 5.69-5.86 and 5.56-5.68 log CFU/g, respectively. The counts of all pathogens were < 10 CFU/g after 2 days, regardless of product formulation. In addition, pathogens were not detected for 3 consecutive analyses in all 5 formulations.

Significance: Salad dressing formulations successfully demonstrated a 5- log reduction of *L. monocytogenes, Salmonella* spp. and *E. coli* O157:H7 within 2 days when stored at ambient temperature (73-77°F). This data suggest that the product needs to be held at ambient temperature for at least 2 days before shipping to ensure safety against foodborne pathogens to meet the draft guidance for Industry for acidified foods.

P2-05 Effect of Thermal Processing on the Survival of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 during the Baking of Pretzels and Pita Chips

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed low moisture foods highlight the need for food industries to validate their thermal process.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli* O157:H7 during the baking of pretzel and pita chips.

Methods: Four dough types with varying water activities (0.88-0.99) were inoculated with *Salmonella* spp. and *Listeria monocytogenes* in separate trials and baked at 300°F and 350°F for 5 to 30 minutes. Least squares regression was used to estimate the baking parameters required to achieve a minimum of 5-log reduction in *Salmonella* spp. and *L. monocytogenes*. These baking parameters were then used to challenge dough samples inoculated with *E. coli* O157: H7. All the experiments constituted 3 replicates. Samples inoculated with *Salmonella* spp., *L. monocytogenes*, and *E. coli*O157:H7 were enumerated using scientifically valid methods. Samples were also analyzed for water activity.

Results: Modeling of pretzel and pita chips data indicated that the thermal processing parameters required for a minimum of 5-log reduction in *Salmonella* spp., *L. monocytogenes,* and *E. coli* O157:H7 during baking were 350°F (hot air temperature) for 19.6 minutes. Water activities ranging from 0.605 – 0.927 and product temperatures ranging from 192 - 230°F were found to be the corresponding food safety verification parameters. The adjusted R-square values ranged from 0.78-0.99 (P< 0.05).

Significance: The study findings provide scientific basis that the thermal process employed by ConAgra Foods bakery manufacturing facilities achieves an acceptable lethality of vegetative pathogens from a food safety standpoint. Because this study indicates that production of a saleable product will result in achievement of adequate food safety parameters, this work supports the management of baking as an operational pre-requisite program (not a CCP) in a facility's food safety plan.

P2-06 Microbiological Growth Profile of *Staphylococcus aureus* and *Bacillus cereus* in Flour-based Batters during Routine Manufacturing Conditions

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Introduction: Flour-based batter systems (a_w > 0.91) used for bakery products may support the growth of *Staphylococcus aureus* and/or *Bacillus cereus* and, given favorable conditions, may produce heat stable enterotoxin during routine manufacturing.

Purpose: The purpose of this study was to evaluate the role of competitive inhibition and the microbiological growth profile of *S. aureus* and *B. cereus* in batters.

Methods: Batter samples were individually inoculated in triplicate with strains of *S. aureus* and *B. cereus* to achieve a target level of 10^2 to10^3 CFU/g. Following inoculation, samples were stored at 20 or 35°C and analyzed at several time points between 0 and 72 hours. Batter samples were analyzed for *S. aureus*, *B. cereus*, and lactic acid bacteria (LAB) using scientifically valid methods. In accordance with FDA published guidance, the food safety limit was defined as 10^5 CFU/g for *S. aureus* and 10^6 CFU/g for *B. cereus*.

Results: At a temperature of 20°C for 72 hours, neither *S. aureus* nor *B. cereus* reached the food safety limit. At a temperature of 35°C, both S. *aureus* and *B. cereus* reached the FDA prescribed limits of food safety around 16 hours. However, both *S. aureus* and *B. cereus* decreased ($P < 0.05$) after 24 hours to below the FDA prescribed limits for food safety. The observed trends at both temperatures were attributed to the growth of naturally occurring LAB.

Significance: Data for *S. aureus* and *B. cereus* suggest the important role of 'competitive inhibition' from naturally occurring LAB at 20 and 35°C in flour-based batters. The study findings indicate competitive inhibition resulted in no significant food safety risk at a temperature of 20°C and a reduction in *S. aureus* and *B. cereus* growth at 35°C. Further investigation of the toxigenic ability of these strains at 35°C is warranted. The data generated in this study provide scientific basis to support manufacturing processes for flour-based batters.

P2-07 Growth Inhibition of *Salmonella enterica* and *Listeria monocytogenes* by Probiotics during a Simulated Gastrointestinal Passage

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Developing Scientist Competition

Introduction: *Salmonella enterica* and *Listeria monocytogenes* are bacterial pathogens of public health significance. Their major route of transmission is through the consumption of contaminated food. Earlier research indicates that regular consumption of probiotics could improve host gastrointestinal defenses.

Purpose: This study was undertaken to determine the fate of *S. enterica* and *L. monocytogenes* as influenced by the presence of probiotics during a simulated gastrointestinal passage.

Methods: A 3-strain mixture of *S. enterica* or *L. monocytogenes* was co-inoculated with a commercial probiotic product into homogenized peanut butter at *ca*. 5 log CFU/ml. Homogenates inoculated with only the pathogens served as controls. To mimic gastric digestion, the pH of the samples was reduced to 1.4-1.9 and pepsin and lipase were added. Samples were incubated for 2h at 37°C. Afterwards, the pH of the samples was increased to 4.3-5.2 and pancreatin and bile were added. Samples were incubated as described above to simulate digestion in the upper small intestine. Finally, the pH of the samples was adjusted to 6.7-7.5 to simulate conditions in the lower small intestines and incubation was done at 37°C for 20h. Aliquots were collected periodically for bacterial enumeration.

Results: Average *Listeria* and *Salmonella* populations in samples without probiotics were higher than the populations co-inoculated with probiotics. After the 24h incubation period *Listeria* and *Salmonella* populations in the controls increased by 5.10 and 5.12 log CFU, respectively, while the two populations in samples with probiotics decreased by 2.30 and 1.34 log CFU, respectively. However, pathogen populations in samples with or without probiotics at sampling points prior to 24h were not significantly different ($P > 0.05$). Additionally, the average populations for the two pathogens in samples with probiotics were not significantly different ($P > 0.05$).

Significance: The survival and growth of *Salmonella* and *Listeria* could be controlled by the presence of probiotics in gastrointestinal system.

P2-08 Thermal Tolerance of O157 and non-O157 Shiga Toxigenic Strains of *Escherichia coli*, *Salmonella* spp. and Potential Pathogen Surrogates in Frankfurter Batter and Ground Beef of Varying Fat Levels

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Developing Scientist Competition

Introduction: Non-O157 Shiga toxigenic *Escherichia coli* (STEC) serogroups most commonly associated with illness are O26, O45, O103, O111, O121, and O145.

Purpose: Thermal tolerance ($D_{55°C}$) of three strains of six non-O157 STEC serogroups was compared with five O157:H7 STEC strains in 7% fat beef. $D_{55°C}$ was determined for > 1 heat-tolerant STEC strain per serogroup in 15 and 27% fat beef. $D_{55°C}$ of single-pathogen cocktails of O157- and non-O157 STEC, *Salmonella* spp., and potential pathogen surrogates, *Pediococcus acidilactici* and *Staphyloccous carnosus*, was determined in 7, 15, and 27% fat beef and in frankfurter batter.

Methods: Samples (25 g) were heated for up to 120 min at 55°C, rapidly cooled, stomached, and plated. Log CFU/g of survivors was plotted versus time and $D_{55°C}$calculated.

Results: There was significant difference in $D_{55°C}$ across all STEC strains in 7% fat beef ($P < 0.05$), but none of the non-O157 STEC strains were more heat tolerant than the range for O157 STEC. There were significant differences in $D_{55°C}$ within serogroups O45, O145, and O157 ($P < 0.05$). $D_{55°C}$-values for non-O157 STEC strains heated in 15 and 27% fat beef were < the range for serogroup O157 ($P \geq 0.05$). $D_{55°C}$ of pathogen cocktails was not different in 7, 15, and 27% fat beef ($P > 0.05$). $D_{55°C}$ of *Salmonella* spp. in frankfurter batter was significantly less than for O157 and non-O157 STEC ($P < 0.05$). Thermal tolerance of pathogens in ground beef (7, 15, or 27% fat) and frankfurter batter was significantly less than for potential surrogates ($P < 0.05$).

Significance: Results suggest that thermal-processing interventions validated against *E. coli* O157:H7 have adequate lethality against non-O157 STEC, that thermal processing interventions which target *Salmonella* destruction may not be adequate against STEC in all situations, and the use of pathogen surrogates *P. acidilactici* and *S. carnosus* to validate thermal processing interventions in ground beef and frankfurter batter would of limited utility to processors.

P2-09 Emergence of Heat-resistant *Salmonella* and *Escherichia coli* Isolated from Food Products

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Introduction: Foodborne illnesses of microbial origin continue to be a major public health threat. Epidemiology of foodborne diseases is rapidly evolving as newly recognized pathogens increase in prevalence or become coupled with new food products. Of interest, *Salmonella* and *Escherichia*

coli which are associated with the consumption of raw or undercooked meat along with other food vehicles. There is a growing concern with these bacteria as they have developed resistance to various controlling methods and conditions including thermal treatment.

Purpose: The purpose of this study was to determine the D- and z-values of newly isolated *Salmonella* and *E. coli* bacterial strains obtained from local foods (chicken, tahini, and raw meat).

Methods: The isolated *Salmonella* and *E. coli* bacterial strains were heat treated at 55, 60, 65, 70 and 75°C. The colony forming unit (CFU) was recorded for each temperature every minute for 10 minutes. Survival curves were plotted and D-values as well as z-values were calculated and compared using two-sample *t*-test.

Results: All bacterial strains tested were totally resistant to heating at 55°C. *E. coli* strains isolated from two sources of raw meat recorded D_{60} of 7.32 and 28.80; D_{65} of 0.65 and 1.02; D_{70} of 0.50 and 0.11; and D_{75} of 0.38 and 0.11 minutes; respectively. The *Salmonella* strain isolated from tahini had D_{60}, D_{65}, D_{70} and D_{75} of 9.45, 0.49, 0.19 and 0.19 minutes; respectively. The other *Salmonella* strains isolated from poultry samples had D_{60} of 3.64 and 3.00; D_{65} of 0.47 and 0.69; D_{70} of 0.19; and 0.54 and $D_{75}$of 0.13 and 0.46 minutes; respectively.

Significance: This study highlights emerging concerns regarding the safety of certain food products in the Lebanese market such as tahini and poultry. These results suggest further revision of associated processing conditions to ensure compliance with food safety standards and avoid outbreaks.

P2-10 Comparative Study of Human Finger Pads and Porcine Skins Used to Quantify Cross-contamination by Norovirus and *Salmonella* during Glove Application and Food Handling

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Introduction: Foodborne illnesses due to norovirus and *Salmonella* can be caused by food handlers with contaminated hands or gloves.

Purpose: In this study, a porcine skin model for bare-hand contact is compared to the fingers of human volunteers and cross-contamination of foods, food-contact surfaces and gloves with *Salmonella* and norovirus is quantified.

Methods: Human fingers (n = 10 volunteers) and sections of porcine skin (n = 5) were inoculated with ~ 5 log of either an avirulent strain of *Salmonella enterica* serovar Typhimurium (χ3985 Δcrp-11 Δcya-12) or Murine norovirus (MNV-1). After a 2-min dry time, fingers or skins were used to touch stainless steel surfaces, latex or nitrile gloves and leaves of sequentially-touched iceberg lettuce (n = 10). Bacteria and virus recovered from recipient surfaces were quantified by real time RT-qPCR (MNV-1) or standard bacterial culture on TSA and percent transfer rates were calculated.

Results: Transfer rates of *Salmonella* from human fingers or porcine skins to stainless steel were 17% and 38%, respectively. *Salmonella* transfer rates to thick latex, thin latex and nitrile gloves from human fingers (21%, 16% and 4%) were lower than the transfer rates from porcine skins (40%, 27% and 19%). Similar findings were observed for MNV-1 to latex glove surfaces, with transfer rates around 25% from human fingers but ranging from 31% – 52% from porcine skins. For each replicate, *Salmonella* was detected on the tenth lettuce leaf sequentially interfaced with contaminated porcine skins, but no *Salmonella* could be detected after the ninth leaf touched with human fingers. For both human fingers and porcine skins, MNV-1 was detected on all of the tenth sequentially-touched lettuce leaves.

Significance: In general, *Salmonella* and norovirus transfer to produce, food contact surfaces and gloves was overrepresented by porcine skins, making it a conservative model for pathogen cross-contamination after bare-hand contact.

P2-11 Identification of Virulence Gene Marker Combinations Influencing the Outcome of pSTEC Testing

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Introduction: FSIS currently declares seven serotypes of pathogenic Shiga toxin-producing *Escherichia coli* (pSTEC) as adulterants of raw non-intact beef products and product components in the USA. Testing relies on the molecular detection of *stx*, *eae*, and a pSTEC O serotype. *E. coli* other than pSTEC can carry various combinations of these target genes, and may lead to a positive screening test (potential positive), but failure to confirm pSTEC.

Purpose: The purpose of this work was to investigate virulence gene combinations in *E. coli* from potential positive samples where pSTEC were not confirmed.

Methods: A total of 121 enrichment broths comprising 93 Big6 and 28 *E. coli* O157 potential positives were analysed using immunomagnetic separation and colony hybridisation targeting *E. coli* that harbor *stx*, *eae*, or were of a pSTEC serotype.

Results: STEC, *eae*-containing *E. coli*, and *E. coli* of a pSTEC serotype that lack *stx* and *eae* were found in 43.0, 28.0 and 28.0% of Big6 samples and 21.4, 14.3 and 3.6% of *E.* coli O157 samples, respectively. Forty-five of 121 (37%) samples were shown to contain multiple isolates of *E. coli* harboring different genetic markers that would be detected during screening and which could account for the potential positive status of that sample.

Significance: This study indicates that organisms other than pSTEC contribute to the generation of potential positive samples during screening. The presence of these organisms may give rise to an elevated potential positive rate, increase the difference between potential positive and confirmed positive rates, and reduce the perceived effectiveness of currently established confirmation protocols. Whilst ongoing refinement of confirmation protocols is required to ensure maximum isolation rates are achieved from samples containing pSTEC, a focus should remain on identifying genetic and or phenotypic differences of pSTEC that can be exploited during the screening process.

P2-12 Characterization of the Virulence Plasmid p7v Harbored in an STEC/ETEC Hybrid Pathotype *Escherichia* sp. Isolate

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Introduction: *Escherichia coli* harboring virulence genes from more than one pathotype have been isolated and these hybrid pathotypes may give rise to new emerging pathogens, such as the case of the 2011 German EAEC/STEC O104:H4 strain. Hybrid STEC/ETEC isolates, encoding both Shiga toxin and heat stable enterotoxin, have been isolated from multiple sources, but have not been well studied. Virulence genes can be transferred between strains via mobile genetic elements such as plasmids; therefore, characterization of plasmids carried by hybrid pathotypes is important for elucidating the evolutionary processes involved in the emergence of new pathotypes.

Purpose: The objective of this study was to characterize the novel virulence plasmid carried by the Shiga toxin-producing *Escherichia* sp. cryptic lineage I O2:H25 strain 7v that is known to also encode heat stable enterotoxin.

Methods: Plasmid DNA was extracted from *Escherichia* sp. 7v and sequenced. Contigs were joined using PCR where necessary to yield a continuous sequence. Phylogenetic analysis utilizing more than 200,000 backbone SNPs from whole genome sequences was performed to place the 7v strain in context with other STEC/ETEC hybrid pathotype isolates as well as prototype strains from additional representative *E. coli* pathotypes.

Results: Plasmid p7v is 229,275 bp in size, contains 340 coding sequences, and harbors the *astA* gene encoding the EAST1 toxin as well as two copies of the gene encoding heat stable enterotoxin STa. Virulence genes more typical of STEC strains, including *katP, ehxA,* and *espP*, are also carried on p7v and, in addition, sequence analysis revealed regions encoding conjugative transfer and several metabolic processes. Phylogenetic analysis revealed a wide diversity of genomic backbones associated with hybrid STEC/ETEC isolates.

Significance: Characterization of strains at the STEC/ETEC pathotype intersection may aid in more rapid detection, identification, and outbreak risk assessment of these emerging threats to the food supply and human health.

P2-13 Genetic Diversity and Virulence Potential of Shiga-toxigenic *Escherichia coli* Strains of O113:H21 Serotype Isolated from the Environment and Foods from Various Countries

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) that cause severe diseases most often also produce the intimin binding protein that enables them to attach to intestinal cells. However, O113:H21 strains do not produce intimin, but have caused hemolytic uremic syndrome (HUS) in Australia and elsewhere. O113:H21 strains have been isolated from the environment in many countries and also from foods and fresh produce. But it's uncertain if these strains are closely related or are pathogenic.

Purpose: Sixty-five clinical, environmental and food isolates of O113:H21 from Argentina, Brazil, France, Germany and the U.S. were compared to the HUS-causing pathogenic strains from Australia to determine if they may be pathogenic. The strains were also examined for genetic diversity.

Methods: A Microarray was used to test for 41 STEC traits. The specific *stx* subtype they carried was determined by PCR. Multilocus Sequence Typing was used to examine clonal relations and genetic diversity was determined by Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) sequence polymorphisms.

Results: All the environmental strains were O113:H21 and indistinguishable from the known pathogens. Most strains carried Stx2a or Stx2c, but a few also had Stx2d or combinations of these subtypes. Stx1a was also found in 14 strains, mainly bovine isolates from Brazil. Majority of the strains had sequence type (ST) 223 or had ST that is closely related to 223. Five strains, 3 from Brazil and 2 from Germany, had ST846 and belonged in a different clonal group. The Australian strains had ST820 that differed from ST223 by a single mutation but are within the STEC-2 clonal group. Although in the same clonal group, CRISPR analysis showed considerable genetic sequence diversity among the O113:H21 strains.

Significance: Environmental O113:H21 strains from the various countries are indistinguishable from the pathogenic strains suggesting that they may also be pathogenic. Most belong in the same clonal group, but are genetically diverse.

P2-14 Virulence of Shiga Toxin-producing *Escherichia coli* under Chlorine Stress

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a major food safety concern that causes outbreaks of severe foodborne illness. It has been suggested that environmental stresses may induce an increased virulence in STEC cells.

Purpose: The purpose of this study was to evaluate the effect of sub-lethal chlorine stress on the virulence of STEC.

Methods: One strain of O157:H7 and 6 strains of non-O157 STEC (O26:H11, O103:H1, O104:H4, O111:NM, O121:NM, and O145:NM) were subjected to four consecutive 24h-exposures to chlorine stress (2 and 5 ppm). The surviving cells were selected as resistant strains. Cells without chlorine stress (control) and with chlorine stress were stored at -80°C until use. Real-time PCR was used to quantify *stx1* gene in each strain. Lactate dehydrogenase assay was performed to determine cytotoxicity in Vero cells. Cytotoxicity levels (%) of cells were calculated and compared using Student's *t*-test.

Results: Results showed that stressed O103:H1, O104:H4, and O145:NM had relatively higher ($P < 0.05$) gene expression of *stx1* and cytotoxicity levels than the control, whereas stressed O157:H7 exhibited only higher cytotoxicity level than the control. For example the cytotoxicity levels (%) were 50.50 ± 5.41 vs. 67.99 ± 5.91 in control and cells of O103:H1 subjected to 2 ppm chlorine stress, respectively. Similar results were observed for O104:H4 (50.17 ± 5.60 vs. 67.21 ± 4.57, respectively) and O145:NM (50.51 ± 4.33 vs. 59.47 ± 2.31, respectively), indicating that chlorine stress induce increased virulence in the selected strains.

Significance: Data from this study suggest that multiple exposures of STEC strains to chlorine stress at sub-lethal concentration may enhance the gene expression of Shiga toxin and its extracellular production. The information will be useful for designing intervention strategies against STEC in food processing plants.

P2-15 Growth Characteristics of Non-O157 Shiga Toxin-producing *Escherichia coli* under Stressed Conditions Induced by Sodium Hypochlorite, pH and Sodium Chloride

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Introduction: Among Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* O157:H7 has been responsible for majority of foodborne illnesses. Recently, non-O157 STEC have been increasingly implicated in foodborne illnesses caused by STEC. It has been shown that environmental stresses could select resistant O157 cells and allow them to persist in the food processing environment. However, the survival behavior of non-O157 STEC in stressed environments is not well understood.

Purpose: The purpose of this study was to examine the effect of environmental stresses on survival of non-O157 STEC.

Methods: One strain of O157:H7 and six strains of non-O157 STEC (O26:H11, O103:H1, O104:H4, O111:NM, O121:NM, and O145:NM) were subjected to three stresses: 8 log CFU/ml of each strain were exposed to chlorine (0, 2, and 5 ppm) at 22°C for 24h; 3 log of each exposed to water activity (a_w 0.95, 0.96, 0.97, and 0.98) or pH (4, 5, 6, and 7) at 12°C for 3 weeks. Survivor curves were plotted and compared using Student's *t*-test.

Results: There were significant differences ($P < 0.05$) in the surviving populations of STEC during stress exposures. Higher populations were observed for O26:H11, O103:H1, and O145:NM strains than others including O157:H7. For instance, after 24h in 2 ppm chlorine, survived cells of O26:H11 and O103:H1 were 6.1 and 5.6 log CFU/ml, respectively, while other strains were less than 3 log CFU/ml. Likewise, 2.2 log CFU/ml of O103:H1 survived for 7 d at pH 4 and 5.6 log CFU/ml of O145:NM survived for 21 d at a_w 0.97, while other strains were not detected or less than 3 log CFU/ml.

Significance: The data suggest that O26:H11, O103:H1, and O145:NM are more resistant to environmental stresses than O157:H7. This information will provide insight into the growth characteristics of non-O157 STEC from O157.

P2-16 Genetic Characterization of Plasmids in O157:H7 and Non-O157:H7 Shiga Toxin-producing *Escherichia coli* Isolated from Humans and Foods

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are responsible for several food and human borne outbreaks worldwide. In the US, O157-STECs cause approximately 90% of hemolytic uremic syndrome (HUS) cases, while ~30% of humans infected with non-O157:H7 STEC develop HUS and hemorrhagic colitis.

Purpose: This study evaluated the Shiga toxins, plasmid profiles and incompatibility groups in STECs.

Methods: 72 STECs (13 O157:H7 and 59 non-O157:H7) from humans and foods were analyzed. Non-O157:H7 STEC variants included O8:H8, O103, O121, O123, O174, O45, O69, O5, O8, O80, O165, O111 and O26. A PCR-based replicon typing scheme was used to detect the presence of IncA/C, B/O, Frep, FIA, FIB, FIC, FIIA, HI1, HI2, I1, K/B, L/M, N, P, T, W, X, and Y. Additionally, Shiga toxin genes (*stx1* and *stx2*) were detected in these isolates using PCR and ELISA.

Results: Data indicated that ~60% of the isolates harbored either the *stx1* or *stx2* gene and 40% harbored both genes. Both genes remained undetected in only one isolate (EC O26). Nearly 30% of O157:H7 isolates carried 1 to 3 plasmids (~0.2 to 100 kb), while 93% of the non-O157:H7 isolates carried 1 to 11 plasmids (~0.25 to 165 kb). Replicon typing data indicated that IncFIB plasmids were detected in 92% of O157:H7 and 76% of non-O157:H7 STEC isolates, while 40% of non-O157:H7 STEC isolates carried IncB/O plasmids. Plasmids for IncB/O, IncFIC, IncP and IncFIA were detected in 8 to 16% of O157:H7 STEC isolates, while IncP, IncK/B, IncI1 and HI2 plasmids were identified in 3 to 7% in non-O157:H7 STEC isolates. Interestingly, only one clinical isolate, EC O111, carried IncX, IncN and IncL/M plasmids.

Significance: STECs harboring plasmids have a greater propensity to cause infections and spread diseases in humans because of the ability these plasmids to encode for multiple virulence, antimicrobial resistance and transfer-associated genes.

P2-17 Differential Distribution of SNP Lineages in Australian and U.S. *Eschericha coli* O157 Isolates

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Introduction: *Escherichia coli* O157 is a foodborne pathogen that has been isolated from many different countries. Recent analyses have used single nucleotide polymorphisms (SNPs) to classify *E. coli* O157 isolates into specific lineages that are associated with animals (termed bovine-biased lineages) and humans (termed clinical lineages), but little is known about genetic variation between different countries.

Purpose: Here, we investigate the distribution of cattle and human associated SNP lineages in *E. coli* O157 isolates from Australia and the U.S.A.

Methods: Ninety-eight *E. coli* O157 isolates (comprising 24 cattle and 25 clinical sources from each country) were screened for the presence of SNPs using a 48-plex Golden Gate assay system which was subsequently used to assign isolates to 11 SNP lineages.

Results: *E. coli* O157 isolates from both countries largely segregated into different SNP lineages. The predominant SNP lineages observed in the U.S.A. were also present in Australia, albeit at much lower frequencies. In contrast, the predominant SNP lineages in Australia (IVb; 48% and IVcvar; 30%) were not detected in this U.S.A. isolate set. The IVcvar SNP lineage represents a unique variant that appears to be exclusive to Australia. Overall, a significantly ($P < 0.05$) higher proportion of U.S.A. isolates (72%) belonged to clinically defined SNP lineages than Australian isolates (4%).

Significance: This study supports previous suggestions that very few Australian *E. coli* O157 isolates belong to the clinical types observed in the United States. The discovery of a new SNP lineage (IVcvar) in Australian isolates suggests that the genotypic diversity of *E. coli* O157 may differ worldwide. Therefore, development of future genotyping methods would benefit from using a globally sourced *E. coli* O157 strain set to ensure that maximum genotypic diversity is captured.

P2-18 Use of Phenotypic MicroArray to Determine Culture Conditions that Regulate the Production of Shiga Toxin in Different *Escherichia coli* Pathotypes

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) include many serotypes with different levels of pathogenicity to humans. The toxins produced by STEC (Shiga toxin 1 and 2), and their subtypes, are one of their major pathogenic attributes. Ultraviolet light, certain antibiotics, and low iron levels have been shown to affect toxin production, but little is known about how other growth and environmental factors regulate the *stx*genes.

Purpose: To compare the effect of different environmental factors on the expression of Shiga toxin among a broad spectrum of STEC to help determine which strains are more pathogenically relevant.

Methods: We are using the Phenotypic MicroArray (PM) developed by Biolog to determine the effect of more than 1,200 culture conditions on the production of Shiga toxin. These conditions include different metabolites such as: carbon, phosphorus, nitrogen and sulfur sources, as well as, antibiotics, chemicals, pH and ionic conditions. The changes in Shiga toxin production were determined quantitatively from PM plate supernatants, by their cytotoxic effect on Vero cells.

Results: Our preliminary results show that the growth conditions that regulate Shiga toxin in *E. coli* O157:H7 and the 2011 German outbreak strain *E. coli* O104:H4 are similar, but some differences have been observed and need to be pursued further. Several carbon sources showed an effect on toxin production including arabinose, ribose, lyxose, xylose and glucosamine to name few. Also, the role on induction of a variety of antibiotics, some consistent with previous studies like ciprofloxacin and others new was identified.

Significance: These studies can lead to the identification of conditions that will allow for a more specific detection and identification of STEC in the food supply.

P2-19 Phenotypic Features of Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups Based on Metabolism

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Introduction: Current techniques for detection of STEC are mostly based on serological and molecular features. However, isolation is not always as successful as detecting them. There is not enough knowledge with regards to the specific nutritional requirements or selective agents in media

that may affect the growth of the different STEC serogroups. Therefore an investigation into effective cultural techniques for the seven regulated STEC serogroups is needed.

Purpose: To identify metabolic characteristics and differences between *Escherichia coli* O157 and the big six non-O157 STEC serogroups that can be used to understand their growth requirements.

Methods: Two different strains of each STEC serogroup were evaluated, including: O157, O26, O111, O103, O45, O121, O145, and O121. The phenotype microarray system from Biolog Inc. was used. Each strain was grown overnight in TSB and colonies were isolated on TSA. Microtiter plates were inoculated by preparing cell suspensions using Biolog protocols. Approximately 760 phenotypic attributes were tested including carbon, nitrogen, sulfur, phosphorous, and peptide nitrogen sources, as well as osmolytes.

Results: Selective STEC serogroup growth was found using the following components: D-Saccharic Acid, D-Serine, D-Sorbitol, D-Ribose, L-Rhamnose, Sucrose, Mucic Acid, m-Hydroxy Phenyl Acetic Acid, L-Galactonic Acid-g-Lactone, b-Methyl-D-Glucuronic Acid, L-Sorbose, b-Hydroxy Butyric Acid, and L-Cysteine. Differences among serogroups were found for the various nutritional components tested. In addition, tolerance to stressors by all STEC serogroups was found to be: NaCl <7%, Urea<6%, Sodium lactate <10%, Sodium formate <5%. Some components that inhibited the growth of all serogroups include: D-Aspartic acid, glycogen, inulin, pectin, urea, and histamine, among others. A comprehensive list of substances that favor or limit their growth was created.

Significance: These results will contribute to our understanding of the STEC serogroups, specially their nutritional requirements to further improve enrichment and solid media, develop new media, enhance the ability to isolate strains, and improve the public health.

P2-20 Stress and Virulence Gene Response of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) Exposed to Low Temperature

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important foodborne pathogens with the majority of non-O157 STEC infections attributed to six serotypes: O26, O45, O103, O111, O121 and O145. Little is known about the fitness and stress response in non-O157 serotypes rendering it difficult to draw conclusions about their behaviour under adverse environmental conditions.

Purpose: To examine the impact of low temperature (4° C) on stress and virulence gene expression in non-O157 STEC.

Methods: Logarithmic phase cultures of *E. coli* O26, O103, O104, O111, O145 and O157 were exposed to 4°C for 7 d. Expression of virulence (*stx1A*, *stx2A* and *eae*), cold stress (*cspA*, *cspC* and *cspE*) and stress response (*rpoS* and *uspA*) genes was examined using a two-step reverse-transcription comparative quantitative real-time PCR. Data was analyzed using Biogazelle's qbasePLUS (ANOVA, significance $P < 0.05$, cut-off at 2-fold difference in gene expression).

Results: *CspA* (cold-shock protein A) was upregulated ($P < 0.05$) in all serotypes, while *cspC* and *cspE* were not differentially expressed. Expression of *cspA* was 5 to 25 fold higher in *E. coli* O157:H7 than all other serotypes. Cold stress slightly increased (<3.5 fold) the expression of *eae* (attaching and effacing) in all serotypes in which the gene was present, but expression of *stx1A* (Shiga toxin) was unaffected ($P > 0.05$). St*x2A* showed slight upregulation in *E. coli* O104:H4, but the difference in expression fell below the 2-fold cut off. Variable gene expression was observed for both of the stress response genes, with *rpoS* showing slight upregulation compared to *uspA*, but neither were differentially expressed from the control.

Significance: The results of the study provide a basic understanding of cold temperature acclimatization of non-O157 STEC, thereby providing knowledge which can be used to develop effective mitigation strategies for their control.

P2-21 *Escherichia coli* O157:H7 Infection Down-regulates Host Autophagy in HT-29 Cells

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Introduction: *Escherichia coli* O157:H7 is a major foodborne pathogen that causes life-threatening hemolytic–uremic syndrome (HUS) and even death. Autophagy is a pivotal catabolic process that degrades unnecessary proteins and intracellular organelles by activating autophagosomes. It also functions to trap invasive bacteria or virulence factors produced by pathogens for clearance. However, little is known about its roles of autophagosomes in *E. coli* O157:H7 epithelial infection.

Purpose: To explore the potential effect of *E. coli* O157:H7 infection on autophagy and apoptosis in host cells

Methods: HT-29 cells were cultured for 24 hours and sampled 4h post *E. coli* O157:H7 infection and then conducted with immuno-blotting and immunofluorescent staining assays.

Results: Both Western blotting and immunofluorescent staining showed a significant decrease of LC3B ($P \leq 0.05$), an autophagic marker, in infected cells as compared to uninfected cells. Because JNK regulates autophagy via Bcl-2 phosphorylation, JNK phosphorylation was further examined and found to be decreased in infected cells ($P \leq 0.05$). These data showed the down-regulation of autophagy in infected cells. Consistently, tumor necrosis factor (TNF)-α, a potent activator of JNK, treatment resulted in increased JNK phosphorylation in HT-29 cells, accompanied by elevated LC3B content. In addition, *E. coli* O157:H7 infection exerted an inhibitory effect on apoptosis of HT-29 cells by blocking the cleavage of poly ADP ribose polymerase (PARP).

Significance: The attenuation of autophagy in infected cells likely promotes *E. coli* O157:H7 survival and colonization, which needs to be further studied. Knowing the mechanism will help to understanding pathogen and host interaction and find ways to eliminate *E. coli* O157:H7 contamination related to meat especially beef processing.

P2-22 Discrimination of Stressed and Unstressed Non-O157 Shiga Toxin-producing *Escherichia coli* Serotypes by Fourier Tansform Infrared (FT-IR) Spectroscopy

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Introduction: Fourier transform infrared (FT-IR) spectroscopy represents a rapid, accurate and selective method to identify bacteria based on their biochemical cellular composition. Recently this technique has also been used to examine the effects of antimicrobial treatments on pathogens by detecting and discriminating injured cells. In 2013 non-O157 Shiga toxin-producing *Escherichia coli* (nSTEC) have been associated with 50% of the total STEC foodborne infections in the United States. Little information is available on the effect of sanitizers and stress conditions on nSTEC growth and the use of FT-IR as a discriminant technique between treatment technologies.

Purpose: The objectives of this research were to evaluate FT-IR as a suitable method to discriminate stressed and unstressed nSTEC cells and to develop a system to classify them based on spectral relatedness and different toxins produced.

Methods: Overnight cell cultures were harvested by centrifugation. Pellets suspended in PBS were treated with 100 ppm peroxyacetic acid solutions. After a 5 min exposure time, bacteria were neutralized and harvested by a series of washing steps. An aliquot was placed on a ZnSe window, dried and analyzed with the FT-IR spectroscope. Untreated nSTEC cultures were used as controls. Linear Discriminant Analysis (LDA) was used to process spectra.

Results: LDA was applied to differentiate nSTEC strains based on the spectra obtained. Two district groups were observed between control and treated bacteria. This indicated that FT-IR was able to discriminate stressed and unstressed cells of *E. coli*. Another LDA model was used to differentiate between Shiga toxin 1 and 2 producers and a relatively promising clustering based on the toxin gene present was observed.

Significance: FT-IR may represent a suitable, rapid and economical tool to discriminate nSTEC serotypes and gain an understanding of physiological stresses that occur in cells following antimicrobial treatments.

P2-23 Effect of Acid Adaptation and Acid Shock on the Thermal Tolerance and Survival of *Escherichia coli* O157:H7 and O111 in Apple Juice

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Developing Scientist Competition

Introduction: Gradual exposure to moderate acidic environments may enhance thermal tolerance and survival of *Escherichia coli* O157:H7 in acid and acidified food products. However, limited studies comparing methodologies to induce this phenomenon have been performed.

Purpose: The effects of strain, physiological state, and acidulant on the thermal inactivation and survival of *E. coli* in apple juice were studied.

Methods: $D_{56°C}$ was determined for *E. coli* O157:H7 C7927 and ATCC® 43895™, and *E. coli* O111 at four physiological states: unadapted, acid-shocked (two methodologies used), and acid-adapted cells. The acidulant effect was evaluated by determining $D_{56°C}$ for the C7927 and 43895 strains subjected to acid shock during 18h in trypticase soy broth (TSB) at pH 5 adjusted with hydrochloric, lactic, and malic acids. *E. coli* survival was determined in juice stored at 1 ± 1°C and 24 ± 2°C. Trials were performed in triplicate.

Results: For thermal inactivation, a significant interaction was observed between strain and physiological state ($P < 0.0001$). Highest thermal tolerance was observed for 43895 subjected to acid shock during 18h in TSB acidified with HCl ($D_{56°C}$ = 3.0 ± 0.1 min), and the lowest for acid-shocked C7927 treated for 4h in TSB containing HCl ($D_{56°C}$ = 0.45 ± 0.06 min). Acidulants did not alter the heat tolerance of C7927 ($D_{56°C}$ = 1.9 ± 0.1 min) ($P > 0.05$), but significantly affected strain 43895 ($P < 0.05$); showing the greatest tolerance with malic acid ($D_{56°C}$ = 3.7 ± 0.3 min). A significant interaction between strain, storage temperature, and physiological state was noted during the survival experiments ($P < 0.05$). *E. coli* O111 was the most resistant strain surviving 6 and 23 days at 24 °C and 1°C, respectively.

Significance: These findings may assist in designing challenge studies for this and similar pH-controlled products, where Shiga toxin-producing *E. coli* represents the pathogen of concern.

P2-24 The Role of Biofilms as a Reservoir for *Escherichia coli* in Irrigation Systems

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are responsible for numerous produce-associated outbreaks. Arizona ranks third in the nation in the production of fresh market vegetables, all of which are grown by irrigated agriculture. Several outbreaks have been linked to contaminated irrigation waters; nevertheless, STEC are rarely found in water and thus it is often difficult to determine the source of contamination.

Purpose: To determine the occurrence and levels of STEC in irrigation biofilms to determine their potential role as a reservoir for STEC.

Methods: A total of 1,115 biofilm samples were collected from sprinkler heads/pipes/gaskets, pumps, and canals in Yuma, Arizona during the growing season (October-April). Samples were enriched in buffered peptone water for 24h at 37°C, followed by selection in EC broth for 24h at 44.5°C. Cultures producing gas were subcultured onto mEndo agar plates; darkly pigmented colonies were evaluated using API20E biochemical strips for species identification. Confirmed *E. coli* isolates were tested for the presence of Shiga toxin (*stx1, stx2*) and intimin (*eae*) genes using qPCR.

Results: *E. coli* was isolated from a total of 51 (4.6%) samples; however, none possessed any of the virulence genes. Generic *E. coli* (non-STEC) was found throughout most of the irrigation systems from the canals, to the pumps, to the sprinkler pipes (proximal and distal ends of the fields). Nevertheless, the organism was identified with greater frequency in irrigation canal biofilms (11.1% of samples) than in other areas of the irrigation systems (4.1% of samples).

Significance: There is likely a low risk of contamination of produce from biofilms containing STEC in Yuma, Arizona. It appears that such contamination either occurs in the field or via sporadic contamination of irrigation waters.

P2-25 Attachment and Biomass Formation of Shiga Toxin-producing *Escherichia coli* (STEC) to Stainless Steel at Varying Temperatures

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Introduction: Six serogroups of Shiga toxin-producing *Escherichia coli* (STEC) were termed adulterants with *E. coli* O157:H7 in non-intact beef products in 2012. Understanding the ability of STECs to attach and form biofilms under varying conditions is needed to improve current food safety policies and techniques.

Purpose: The objective of this study was to measure the attachment and biomass formation of STEC O26, O45, O103, O111, O121, O145 and O157:H7 strains on stainless steel coupons over time when incubated at 7°C and 25°C.

Methods: Seven strains previously determined to have a strong affinity for attachment and one strain determined to have a low affinity for attachment were used in this study. Strains were used to inoculate stainless steel coupons in M9 minimal salt media and incubated at either 7°C or 25°C for up to 96h. At 4h, media was removed, all coupons were rinsed with water, and fresh media was added. At the time interval (4, 48 or 96h), the coupons were removed, rinsed, and stained with crystal violet. Crystal violet was removed from the coupon and solution absorbance at 590 nm was measured.

Results: Significant ($P < 0.0001$) interactions for strain*temperature and temperature*hour were noted. Strains of O45, O103, O111, and O157:H7 had differences in absorbance between temperatures. Differences among strains were noted for each temperature. Differences in attachment between temperatures were observed at 48 and 96 hours. At 25°C, attachment increased after 48 hours, while at 7°C, an increase was observed at 48 hours, then absorbance declined at 96 hours.

Significance: These data indicate that the strains tested can attach and form biomasses at both ambient and refrigerated temperatures. However, attachment and biomass formation appears to be strain dependent. Further research is needed to understand if other strains within these serogroups have the ability to attach under similar conditions.

P2-26 Attachment of Shiga Toxigenic *Escherichia coli* (STEC) on Stainless Steel and Polyurethane

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Developing Scientist Competition

Introduction: Biofilm formation can have a negative impact on food quality and safety due to the persistence of pathogens and spoilage bacteria on processing equipment. Shiga toxin-producing *Escherichia coli* (STEC) from serogroups O26, O45, O103, O111, O121, O145, and *E. coli* O157:H7 are adulterants in non-intact beef products. Understanding how these bacteria grow and interact within the food processing environment is important to food safety.

Purpose: The objective was to enumerate attachment and biomass formation to stainless steel (SS) or polyurethane (CB) coupons by strains of each STEC serogroup.

Methods: Three strains from each serogroup with differences in strain origin and presence of *stx1* and *stx2* were examined. Coupons of SS or CB were incubated in minimal or full nutrient broth (TSB) inoculated with a single STEC strain at 25°C for up to 48h. At 4h, loosely attached cells were rinsed from coupons and fresh media was added. At the time interval (4, 24 or 48h), coupons were removed, rinsed, placed in buffered peptone water (BPW) with beads and vortexed. Serial dilutions were plated onto tryptic soy agar and incubated for 24h at 37°C. Data were converted to CFU/cm^2 prior to analysis.

Results: For coupons incubated in minimal media, there were no significant differences ($P > 0.05$) for attachment and biomass development for the coupon material. However, a significant interaction ($P < 0.05$) was observed for strains over time. Coupons inoculated with strains in TSB had significant interactions for strains over time and coupons over time. For both SS and CB coupons, significant increases in biomass formation were observed between 4 and 24h.

Significance: These results show STEC biomass formation are strain dependent. STECs have also been shown to attach and grow on different processing surfaces. Further research to understand efficacy of cleaners and sanitizers against these strains that form biomass is needed.

P2-27 Inactivation of *Escherichia coli* O157:H7 in Biofilm on Wooden Surfaces by Sequential Treatments of Aqueous Chlorine Dioxide and Drying

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Poster

Introduction: Biofilm formation of *Escherichia coli* O157:H7 on the surface of food-cutting boards can be a source of cross-contamination of pathogens into foods. Therefore, effective decontamination procedures to inactivate *Escherichia coli* O157:H7 on the surface of food-cutting boards should be developed.

Purpose: This study was done to evaluate the synergistic lethal effects of sequential treatments of aqueous chlorine dioxide (ClO_2) and drying against *E. coli* O157:H7 in biofilm on wooden surfaces.

Methods: Biofilms of *E. coli* O157:H7 on wooden coupons were produced in minimal salt broth at 22°C for 5 days. Wooden coupons containing biofilms of *E. coli* O157:H7 were treated with 200 µg/ml of ClO_2 or sodium hypochlorite (NaOCl) solution for 10 min, thoroughly rinsed in sterile water, and subsequently dried with 43% relative humidity (RH) at 22°C for up to 12h. The numbers of *E. coli* O157:H7 survived on wooden coupons after treatment of ClO_2or NaOCl solution and drying were measured.

Results: *E. coli* O157:H7 was able to form biofilms on wooden coupons (9.5 log CFU/coupon). When the coupon containing the biofilm was treated with NaOCl solution, the population of *E. coli* O157:H7 on coupons decreased to 8.7 log CFU/coupon. The subsequent drying caused 1.0 log CFU/coupon of additional reduction. When coupons containing biofilm were treated with ClO_2 solution, the number of *E. coli* O157:H7 on coupons decreased to 7.6 log CFU/coupon. The subsequent drying decreased the number of the pathogen on coupons by an additional > 7.0 log CFU/coupon.

Significance: Sequential treatment of aqueous ClO_2 and drying showed synergistic lethal effects in inactivating *E. coli* O157:H7 in biofilm on wooden surface.

P2-28 *Escherichia coli* O157:H7 Biofilm Formation by MBEC™ Assay: Effect of Curli Expression on Its Resistance to Natural Antimicrobials

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Introduction: *Escherichia coli* O157:H7 biofilm formation is a concern because sessile bacteria within biofilms are not easily killed by treatment with antimicrobials. Natural antimicrobials have been evaluated as a produce wash to remove pathogens from fresh produce.

Purpose: The purpose of this study was to evaluate the effects of natural antimicrobials in reducing cells in biofilms formed by curli-expressing *E. coli* O157:H7 strains.

Methods: Three morphotypes of *E. coli* O157:H7 86-24: wild-type, curli deficient 86-24D*csgA* mutant, and curli over-expressing constitutive 86-24*csgD*^{c} mutant strains were used in MBEC™ HTP assay to study biofilm formation. Following incubation in LB medium w/o salt for 48h at 25°C, biofilm formed on MBEC™ polystyrene pegs (n = 36) were analyzed by crystal violet assay. Biofilms were treated with cinnamaldehyde and Sporan at 1000-3000 µg/ml or chlorine (50 µg/ml) for 10 min to evaluate their potentials in removing biofilm. Following neutralization of antimicrobials, surviving *E. coli* O157:H7 populations in biofilms were dislodged by sonication and then analyzed by spiral plating on SMAC media.

Results: The biofilm formation by *E. coli* O157:H7 strongly correlated with their ability to produce curli. The curli over-expressing mutant produced significantly ($P < 0.05$) greater biomass (0.861 ± 0.046) compared to wild-type (0.296 ± 0.018) and curli-deficient strain (0.115 ± 0.010). Cinnamaldehyde and Sporan significantly reduced *E. coli* O157:H7 (3-5 log CFU reductions) in biofilm. The efficacy of antimicrobials in reducing cells in biofilm was increased with an increase in their concentrations. Chlorine was superior to natural antimicrobials in reducing cells in bioiflms. Cells in biofilm formed by curli over-expressing strain showed greater resistance to antimicrobials than cells formed by wild or curli-deficient strains.

Significance: Cinnamaldehyde and Sporan have potential in reducing *E. coli* O157:H7 in biofilm. Biofilm formed by curli over expressing *E. coli* O157:H7 may require higher antimicrobial concentrations to remove cells from equipment surfaces.

P2-29 Presence of Extra-intestinal Pathogenic *Escherichia coli* (ExPEC) in Agricultural Environments

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Introduction: Extra-intestinal pathogenic *Escherichia coli* (ExPEC) have a combination of virulence factors which allow them to cause disease in both humans and animals. Little is known about their persistence and survival in agricultural environments. If present in agricultural environments, it may be possible for ExPEC to contaminate produce commodities and cause human infections through produce consumption. There is no defined criterion for classification of an *E. coli* isolate as an ExPEC other than the presence of a single ExPEC virulence factor.

Purpose: This study determined if ExPEC were present in commercial finished compost, indicating their presence in agricultural environments.

Methods: Seventy-seven *E. coli* isolates from finished commercial compost were assayed by polymerase chain reaction (PCR) for the presence of nine virulence factors (VF) commonly associated with Avian Pathogenic *E. coli* (APEC) and ExPEC using previously designed primers: *astA* (enteroaggregative toxin), three *iss* alleles (serum resistance), *papC* (P fimbriae), *iucD* (aerobactin), *tsh* (hemagglutinin), *vat* (vacuolating toxin), and *cvaA/cvi* (colicin V plasmid).

Results: Only two isolates (3%) of 77 did not contain any ExPEC VF. Thirty-eight (50%) of the *E. coli* isolates contained either two or three ExPEC VFs. Thirty isolates (39%) were positive for at least one adhesin (*papC*, *tsh*) and one toxin (*astA*, *vat*), indicating a minimum threshold to cause infection. Twenty-one isolates (27%) were found to be positive for *papC* or *tsh* and at least one serum resistance allele (*iss* types 1, 2, and 3), also indicating their potential to cause human illness. The two most prevalent virulence factors identified were *astA* (90%) and *iss* type 1 (69%).

Significance: These results show that ExPEC are present in commercial composts, which when used in the growing environment may introduce the pathogen to produce commodities. As more attention is paid to transmission of extraintestinal infections to humans and animals, the presence of ExPEC in agricultural environments deserves attention.

P2-30 Evidence of Transmission of *Escherichia coli* O157:H7 to Tissues or Phyllo-plane of Wheat from Contaminated Soil, Seeds or Water and Survival on Flowering Heads

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Introduction: *Escherichia coli* O157:H7 is a human pathogen associated with raw beef; however, recent outbreaks have linked it to fresh produce and wheat flour products. Studies have shown that *E. coli* O157:H7 can internalize into plant tissues in produce, but there is a lack of knowledge regarding the organism's internalization into wheat throughout the growing season.

Purpose: The objective of this research was to determine the possible transmission route of *E. coli* O157:H7 into the phyllo-plane of wheat using contaminated seed, soil, or water and survivability of *E. coli* on wheat heads.

Methods: A cocktail of five kanamycin-resistant strains of *E. coli* O157:H7 was used to contaminate the seeds, soil or irrigation water with 6.88 log CFU/g, 6.60 log CFU/g and 6.76 log CFU/ml, respectively. Seedlings were harvested after 9 days post-inoculation. A fourth experiment was conducted in which flowering wheat heads were sprayed with contaminated water (4.19 log CFU/ml) and harvested after 15 days. Approximately one hundred plants per experiment were sown, watered every day, surface sanitized (except in the fourth experiment), enriched with selective media, and analyzed by *q*PCR detection kits.

Results: Internalization of *E. coli* into wheat seedlings using contaminated seed, soil, and irrigation water occurred at a rate of 2% (96 samples tested), 5% (100 samples tested) and 10% (100 samples tested), respectively. The wheat head contamination experiment showed the ability of *E. coli* O157:H7 to survive on the plant for at least 15 days (100% survival, 98 samples tested).

Significance: Even though the internalization rate shown was low in seedlings, this is the first study demonstrating the ability of *E. coli* O157:H7 to reach the internal tissues of the wheat phyllo-plane. Furthermore, surface contamination and the ability of *E. coli* O157:H7 to survive long-term on wheat heads should be considered when addressing food safety concerns.

P2-31 Withdrawn

P2-32 Identifying the Accuracy of Non-O157:H7 Shiga Toxin-producing *Escherichia coli* Testing: A Proficiency Testing Study

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Introduction: While testing for *Escherichia coli* O157:H7 has been required for many years, in September 2011 the U.S. Department of Agriculture declared the following non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) strains as adulterants: O26, O45, O103, O111, O121, and O145. Mandatory testing of beef trim for the six additional STEC strains began in June 2012, and testing for these adulterants is novel to U.S. food laboratories. With this mandatory testing comes the need for proficiency testing (PT). The American Proficiency Institute (API), a food PT provider accredited by A2LA to ISO/IEC 17043, offers the only accredited PT program for all seven STEC adulterants.

Purpose: This PT study includes data from three test events in 2013, where 128 participants submitted results for detection and identification of an unknown STEC. Our objective was to assess whether laboratories can properly detect and identify STEC adulterants.

Methods: Responses for the seven STECs were recorded from 254 cumulative proficiency testing results submitted in three test events in the year 2013. Participants were allowed to test the PT sample using the method of their choice and were asked to report the method used.

Results: Laboratories accurately detected an STEC present in a sample 96.1% of the time. When the laboratory attempted to identify the strain, 95.6% of identifications were correct. However, 39.9% of responses indicated participants were unable to, or chose not to, identify the strain in a particular sample.

Significance: As laboratories gain experience in routine testing for these STEC adulterants, proficiency testing will be useful in assessing their ability to detect and identify STECs. The current results indicate that a significant portion of laboratories may test for the presence of STECs without identifying the strain. This may change as industry practices and laboratory test methods for identifying these strains continue to evolve.

P2-33 Effect of Nalidixic Acid Adaptation on Shiga Toxin and Non-pathogenic *Escherichia coli* to Radio Frequency Heating

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Introduction: O157:H7 and non-O157 Shiga Toxin *Escherichia coli* (STEC) are recognized as major foodborne pathogens resulting in serious health issues such as HUS and kidney failure. Radio Frequency (RF) uses electromagnetic energy to heat foods with rapid heat distribution and relatively lower energy consumption. RF heating can be used to reduce high levels of microbial contamination in foods such as beef. Efficacy of new interventions to control STEC that use antibiotic resistant pathogens must be validated for similar resistance to interventions as parent strains.

Purpose: To validate the use of Nalidixic acid- (NA-) adapted strains of three STEC and non-pathogenic *E. coli* in studies of the efficacy of RF heating using a model solution.

Methods: A total of 15 parent strains and 15 NA strains of *E. coli* O157:H7, O26:H11 and O111 along with 4 non-pathogenic surrogates were used on this study. Cultures of each strain were centrifuged and combined in equal proportions to prepare cocktails of respective serotype. Phosphate buffered saline solutions were inoculated with cocktail and heated using an RF oven to three different endpoint temperatures (55°C, 60°C and 65°C) to be appropriately enumerated.

Results: Results showed log reduction between 0.5-1.0 for strains treated at 55°C, between 1.0-1.5 for strains treated at 60°C and above 5.0 for strains treated at 65°C for all serotype cocktails. No significant differences were observed between parent and NA strains for each temperature.

Significance: Results revealed that there was a significant difference in log reduction comparing 55°C and 60°C to 65°C. There was not a significant difference between parent and NA-adapted strains which suggests that NA-adapted strains might be a good choice to substitute the parent strains for RF experiments in beef.

P2-34 From Farm-to-Fork: EMD Millipore Singlepath Direct Campy Poultry Rapid Test Kit for Farm-based Direct Detection of *Campylobacter* spp. in Caecal-type Samples from Live Chicken

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Introduction: The 2012 EFSA Scientific Opinion on meat inspection (EFSA Journal 2012;10(6):2741) proposed testing the *Campylobacter* status of live broiler flocks ≤ 3 days prior to slaughter, to identify the 'high shedding' flocks and allow segregation from low-shedding at slaughter, thereby avoiding cross-contamination of carcasses and reducing human consumption of *Campylobacter* spp. Such a strategy requires on-farm testing and a method which requires no specialized equipment or laboratory-trained personnel. Lateral Flow technology fulfils this requirement and offers a reliable, fast, user-friendly, alternative detection method to the laboratory-based cultural reference methods.

Purpose: To develop and evaluate a qualitative immunochromatographic assay for direct (non-enrichment) detection of high shedding (≥ 7.5 log CFU/g of caecal-type sample) *C. jejuni* and *C. coli* broiler chicken flocks, within 2 hours of sampling, as a rapid and farm-based alternative to standard cultural reference methods to monitor *Campylobacter*status of flocks and assist slaughter scheduling.

Methods: A sandwich Lateral Flow assay was developed, using gold labelled specific antibodies for *Campylobacter* spp. A non-enrichment sample preparation protocol was developed to enable a time-to-result of within 1 hour of sampling. Evaluation was by field studies conducted both on-farm (caecal droppings) and at slaughterhouse (caecal contents) using a cross-seasonal representative set of broiler chicken caecal-type samples. Reference method comparison was with ISO 10272 cultural method.

Results: In a field trial of caecal droppings collected on farm (n = 60), *Singlepath® Direct Campy Poultry* achieved a sensitivity of 96% (% correctly classified positive) and a specificity of > 99% (% correctly classified negative) based on a Limit of Detection of ≥ 7.5 log CFU/g of caecal content. In a field trial of caecal contents collected at slaughter (n = 60), *Singlepath® Direct Campy Poultry*achieved a sensitivity of 96% (% correctly classified positive) and a specificity of > 99% (% correctly classified negative) based on a Limit of Detection of ≥ 7.5 log CFU/g of caecal content.

Significance: EMD Millipore *Singlepath® Direct Campy Poultry* Rapid Test Kit provides a unique, alternative, fast and simple method for detection of high shedding ≥ 7.5 log CFU/g of caecal-type sample) *C. jejuni* and *C. coli* broiler chicken flocks, on-farm or at slaughter, and can assist in monitoring *Campylobacter* spp status of flocks and in slaughter scheduling.

P2-35 Proteomic Insights into the Molecular Response of *Campylobacter jejuni* to Acid Shock

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Introduction: *Campylobacter jejuni* is a leading cause of foodborne bacterial gastroenteritis in humans. As *C. jejuni* transfers from environmental sources into the host gastrointestinal tract, it is exposed to low pH. Survival in an acidic condition may play a critical role on the pathogenesis of *C. jejuni*.

Purpose: The purpose of this study was to identify differentially expressed proteins that were differentially regulated in response to acid shock.

Methods: *C. jejuni* NCTC 11168 was exposed to MH broth buffered at pH 7.4 (control) and pH 4.5 for 1 h at 37°C in a microaerophilic chamber. Proteins from control and low pH were extracted and digested. After trypsin-catalyzed $^{16}O/^{18}O$ stable isotope labeling, the peptides from control and acid shock samples were mixed at a 1:1 ratio. The mixture was then analyzed using two-dimensional liquid chromatography coupled online with an LTQ-Orbitrap XL mass spectrometer for MS/MS.

Results: Proteomic analysis revealed that a total of 726 proteins were identified and, among them, 162 proteins, which accounted for approximately 22.3% of the total proteins identified, were significantly changed (over 1.4-fold, $P < 0.05$) under control and low pH conditions. The differentially expressed proteins (up-regulated, 68 and down-regulated, 94 proteins) are involved in a variety of cellular functions (metabolism, 64, cellular processes and signaling, 26, information storage and processing, 26, and poorly characterized, 13). In 2008, approximately sixty genes of *C. jejuni* required for growth at low pH were identified by microarray technique (Reid et al, Applied and Environmental Microbiology, 2008, 74(5):1583-97). Out of those genes, twenty-five genes were determined as proteins in this proteome study. Four proteins, glucose-methanol-choline oxidoreductase subunit (cj0415), NADH dehydrogenase subunit L (NuoI), NADH dehydrogenase subunit G (NuoG), and citrate synthase (GltA), were significantly expressed at low pH condition, whereas two proteins, iron-uptake ABC transporter substrate-binding protein (CfbpA) and histidinol dehydrogenase (HisD), were significantly expressed at pH 7.4.

Significance: For the first time, we investigated the whole proteomic profile of *C. jejuni* when exposed to low pH. Comparative proteome analysis may provide useful information for the molecular characterization of acid shock response in *C. jejuni*.

P2-36 Biofilm Formation by *Mycobacterium bovis*: Influence of Surface Kind and Temperatures of Sanitizer Treatments for Biofilm Control

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Introduction: *Mycobacterium bovis* has a wide range of hosts including humans and wildlife, making it a pathogen of public health significance. Humans are usually infected with *Mycobacterium* through inhalation of droplet nuclei; however, a significant proportion of human cases involve extrapulmonary TB, presumably caused by the consumption of raw milk from infected animals or food products contaminated with the pathogen.

Purpose: This study was undertaken to assess biofilm formation by selected strains of *M. bovis* on different contact surfaces and in various growth media and to test the efficacy of sanitizing treatments at various temperatures for control of the biofilms formed by *M. bovis* on different contact surfaces.

Methods: Biofilm-forming abilities of two *M. bovis* strains were assessed in 150 ml Middlebrook 7H9-Tween 80 (0.1%) broth with 5% liver extract and 10% oleic albumin dextrose catalase (OADC), 5% liver extract alone, or 10% OADC alone in sterile jars containing 2 x 2 cm^2coupons of steel, cement or ceramic. The jars were incubated at 37°C with agitation for 2, 3 and 4 weeks, respectively. Biofilms on the coupons were subsequently exposed to 10 ml of 2% Iodasteryl®, 0.5% Virocid® and sterile water at 28 and 85°C, respectively for 5 min. Residual biofilms on treated coupons were quantified using the crystal violet binding assay.

Results: The two *M. bovis* strains had a similar ability in forming biofilms on the three surfaces. More biofilms were developed in media containing 5% liver extract. Biofilm mass increased as incubation time increased till the 3rdweek. More biofilms were formed on cement than on ceramic and stainless steel surface. Sanitizing treatments at 45°C removed more biofilms than at 28°C. However, neither treatment completely eliminated the biofilms.

Significance: Choice of processing surface and temperatures used for sanitizing treatments had an impact on biofilm formation and its removal from solid surfaces.

P2-37 The Influence of Polysacharides and Polysaccharide-hydrolyzing Enzymes on Biofilm Formation by *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a pathogen that is responsible for less than 1% of foodborne illness but accounts for 28% of the deaths caused by foodborne pathogens. Formation of biofilms by *L. monocytogenes* is of great concern as this facilitates the persistence of an already ubiquitous pathogen in the environment.

Purpose: This study investigates the effect of 3 polysaccharides at different concentrations and their hydrolyzing enzymes on the formation of biofilms by selected strains of *L. monocytogenes* over a period of 120h.

Methods: Biofilms were developed in polystyrene tissue culture plates by 4 food isolates and 4 disease outbreak isolates of *L. monocytogenes* strains in tryptose soy broth (TSB) with 3 different concentrations (0, 0.02 and 0.04%) of pectin, cellulose or starch and with or without the addition of pectinase, cellulose or amylase at 37°C during a period of 24 to 120h. The biofilms formed were quantified using the Crystal Violet Binding Assay. The influence of polysaccharides, their hydrolyzing enzymes and incubation time on biofilm formation was analyzed at a confidence level of 95% using a multifactorial design of the Statistical Analysis Software.

Results: Presence of external polysaccharides negatively affected biofilm formation. Increase in polysaccharide concentration did not have a significant influence on biofilm formation by the *Listeria* strains. Addition of pectin, cellulose and starch hydrolyzing enzymes led to a significant increase in biofilm mass. Generally, the foodborne isolates (0.171-0.284) formed more biofilms than did the diseases outbreak isolates (0.086-0.100) used in the study. Biofilm formed by the *L. monocytogenes* strains in TSB with or without polysaccharide-hydrolyzing enzymes increased significantly with incubation time starting from the 72h sampling point and forwards.

Significance: The research provides knowledge on how *Listeria* biofilm formation is regulated by external polysaccharides and their hydrolyzing enzymes.

P2-38 Antimicrobial-resistance Pattern and Molecular Mechanism of *Arcobacter butzleri* Isolated from Chicken in Korea

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Introduction: According to recent reports, *Arcobacter* species is an important emerging pathogens which has the similar characteristics of *Campylobacter* species. Recently, outbreak of *Arcobacter butzleri* by the consumption of contaminated food was reported in Wisconsin, USA.

Purpose: The aim of this study was to investigate the antimicrobial pattern and analyze the genomic sequence of quinolone-resistance determinant region (*grdr*) of *gyrase A* in *Arcobacter butzleri* isolates.

Methods: Thirty-nine of *A. butzleri* were isolated from chicken in Korea. The antimicrobial susceptibility of 39 *A. butzleri* isolates and *A. butzleri* ATCC 49616 was performed with 22 antimicrobial agents following the guideline of NCCLS. Also the *qrdr* of the *A. butzleri gyrA* genes were amplified and sequenced.

Results: More than 95% of *A. butzleri* isolates were susceptible for aminoglycosides including amikacin, gentamicin, tobramycin, kanamycin, linsmycin, streptomycin, and neomycin. However, all isolates showed resistance to penicillin, sulfamethoxazole, ampicillin, cephalothin, and vancomycin. Seventeen of *A. butzleri* isolates were resistant to quinolones including enrofloxacin and ciprofloxacin. The quinolones-resistant 17 isolates had point mutation of *qrdr*which substitute Serine at 53 positions into Isoleucine.

Significance: The amikacin, gentamicin, tobramycin, linsmycin, streptomycin, and neomycin could be effective antibiotics to treat *A. butzleri* infection. The point mutation of *qrdr* of *A. butzleri* strains was highly associated with resistance to quinolones.

P2-39 Immunological Detection of *Brucella* Species

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Poster

Introduction: *Brucella* causes brucellosis in animals and humans globally. In particular, pathogenic species like *B. abortus* and *B. melitensis* can be transmitted through milk. For isolation and detection, a BSL-3 laboratory setup and labor intensive culturing method is used. Based on the highly intraspecies homology of *Brucella*, lipopolysaccharides (LPS) of non-pathogenic *B. neotomae* was used as a model antigen to develop antibody and was further tested against pathogenic *Brucella* species.

Purpose: To develop antibody against *B. neotomae* LPS for detection of pathogenic *Brucella* species especially *B. abortus* and *B. melitensis* in milk.

Methods: *B. neotomae* LPS was extracted by butanol-water method, quantified by endotoxin assay kit, tested by silver staining and was used to immunize rabbits. The Protein-A purified antibody was tested in enzyme linked immunosorbent assay (ELISA) and Western blotting. Purified LPS of *B. neotomae*, *B. abortus RB51*, *E. coli* and 6 strains of pathogenic *Brucella* species and their whole cell lysates were used as antigen to evaluate the specificity of the antibody.

Results: In ELISAs, there was significant difference in antibody reaction between *B. neotomae* and *E. coli* O157:H7 LPS, and between *B. neotomae* and whole cell lysates of other Gram negative species such as *Salmonella*. Immunofluorescence absorbance were 8168 ± 117(1:2000 diluted antibody) RFU for *B. neotomae* LPS vs. 264 ± 8 (1:2000) RFU for *E. coli* O157:H7 LPS. In Western blot, 6 strains of pathogenic *Brucella* species (*B. abortus*, *B. melitensis*) showed identical bands as *B. neotomae*, while *B. abortus RB51*, a LPS negative vaccine strain and *E. coli* O157:H7 showed negative result with no bands.

Significance: *B. neotomae* may be used as a model for antibody development and detection of pathogenic *Brucella* species.

P2-40 Anti-apoptotic Effect of Bioactives from *Lactobacillus helveticus* and *Lactobacillus acidophilus* against *Salmonella* Typhimurium Infection on Epithelial Cells

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Introduction: Both *in vitro* and *in vivo* studies have shown that bioactive components produced by *Lactobacillus helveticus* and *Lactobacillus acidophilus* exert a protective effect against infections caused by a variety of enteric pathogens, including *Salmonella*.

Purpose: The goal of this study was to determine the mode of protective effect from bioactive molecules produced by *L. helveticus* (LH-2) and *L. acidophilus* (La-5) against *Salmonella* Typhimurium infection.

Methods: Bioactives were produced after 48 hours fermentation in milk or whey protein-based media for LH-2 and La-5, respectively. Colonic carcinoma HT-29 cells were grown in Transwell inserts for 40 days until polarized (~120 ohm×cm^2). Cell membrane integrity was measured through transephithelial electrical resistance (TEER). The nontoxic doses of bioactives were determined using trypan blue exclusion/SRB assay. Bioactives were pre-incubated 24 h prior to *Salmonella* infection and co-incubated during *Salmonella* infection. Lactate dehydrogenase (LDH) activity was tested using Abcam® LDH-cytotoxicity assay kit. Apoptosis was measured by flow cytometry with BD™ APO-Direct kit. Finally, an invasion assay was carried out using chicken hepatoma LMH cells as an *in vitro* model for *Salmonella* presence in poultry.

Results: The protein concentration of 370µg/ml LH-2 and 1.5mg/ml La-5 were nontoxic for the epithelial cells. LH-2 and La-5 exhibited protective effects of TEER *(P* < 0.05*)*, LDH and apoptosis *(P* < 0.01*)* against *S.* Typhimurium on polarized HT-29 cells. In infected polarized HT-29 cells, LDH was reduced up to 49.1% in presence of LH-2 and up to 46.8% when La-5 was present. In terms of apoptosis, compared with positive control 62.5% and 75.8% less cells underwent apoptosis with LH-2 and La-5 treatment, respectively. *Salmonella* invasion of LMH cells was reduced less than one log cycle.

Significance: These data suggest that LH-2 and La-5 bioactives reduce the amount of cells undergo apoptosis following *Salmonella* infection.

P2-41 Occurrence of *Listeria* spp. in Retail Meat and Dairy Products in the Area of Addis Ababa, Ethiopia

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Introduction: Listeriosis, a bacterial disease in humans and animals, is mostly caused by ingestion of *Listeria monocytogenes* via contaminated food and/or water, or by a zoonotic infection. Globally, listeriosis has in general a low incidence but a high case fatality rate.

Purpose: The objective of this study was to investigate the occurrence, prevalence, and genetic relatedness of *L. monocytogenes* from raw meat and dairy products (raw milk, cottage cheese, cream cake), collected from the capital and five neighbouring towns, in Ethiopia.

Methods: Two hundred forty food samples were purchased from July to December 2006 from food vendors, shops and supermarkets, using a cross-sectional study design. *L. monocytogenes* spp were isolated and subjected to molecular serotyping. The genetic relatedness then investigated using pulsed-field gel electrophoresis (PFGE) technique.

Results: Two hundred and forty food samples tested, 66 (27.5%) were positive for *Listeria* species. Of 59 viable isolates, ten (4.1%) were *L. monocytogenes.* Nine were serotype 4b and one was 2b. PFGE testing of the ten *L. monocytogenes* isolates showed low relatedness among eight different PFGE types.

Significance: The findings in this study correspond to similar research undertaken in Ethiopia by detecting *L. monocytogenes* with similar prevalence rates. Public education is crucial as regards the nature of this organism and relevant prevention measures. Moreover, further research in clinical samples should be carried out to estimate the prevalence and carrier rate in humans, and future investigations on foodborne outbreaks must include *L. monocytogenes*.

P2-42 Isolation of Haemolytic Strains of *Listeria innocua*, in Addis Ababa, Ethiopia

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Introduction: Human Listeriosis is caused by the ingestion of haemolytic strains of *Listeria monocytogenes with* contaminated food and water*.* However, nowadays, natural occurring haemolytic strains of *L.innocua* are currently reported due to the evolution of the virulence factors, and there are few findings which reports hly gene positive *L. innocua* species (atypical form) and also hly gene negative *L. monocytogenes* spp.

Purpose: The objective of this study was to investigate the presence of atypical form of *L. innocua* among the isolates which were previously collected from raw meat and dairy products in Ethiopia.

Methods: Forty nine *L. innocua* species were checked for haemolytic and biochemical reactions using conventional culture techniques. However, there was some disagreement among the sugar fermentation results and the haemolytic reactions. Therefore, the isolates were subjected to further confirmation by molecular typing, using Multiplex PCR, with the amplcon size of 289 bp for *L. innocua*. Then all confirmed species were checked for the presence of haemolytic gene (hly gene).

Results: All 49 isolates collected from Addis Ababa and neighboring towns were confirmed as a typical form of *L. innocua* species, which are non-pathogens, since they don't possess the gene which plays a great role in haemolytic and cell membrane eruption of living cells, and helps the organism to multiply and invade tissues in both intrinsic and extrinsic pathways.

Significance: Unlike few studies on atypical forms of *L. innocua*, this finding proves, all tested *L.innocua* species were non-pathogens, do not posses the hly gene. However, as the result of sharing some common genes among the *Listeria* species and emerging form of atypical forms of *L. innocua,* and *L. monocytogenes* spp., biochemical testing method for the isolation of the species becomes less sensitive, OR creates bias. Therefore, confirmation using high tech is mandatory.

P2-43 Rapid Detection of *Listeria monocytogenes* in a Variety of Ready-to-Eat (RTE) Products from Retail Stores in Thailand

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Introduction: Listeriosis outbreaks have been widely associated with a variety of ready-to-eat (RTE) foods. Faster and reliable results are therefore necessary for early detection of *L. monocytogenes* in RTE products to ensure product safety during shipping and prolonged storage. The 3M™ Molecular Detection Assay (MDA) *Listeria monocytogenes* is a rapid method that uniquely utilizes isothermal DNA amplification and bioluminescence to detect *L. monocytogenes* significantly faster than the traditional culture method.

Purpose: This study evaluated the performance of the 3M MDA *Listeria monocytogenes* for the detection of *L. monocytogenes* in various RTE products as compared to the standard FDA-BAM method.

Methods: Products (n = 200) were purchased from several different sized retail stores. These included 122 aquatic products (from fresh and salt water), 22 products of animal origin (e.g., smoked chicken wings), 18 vegetarian products (e.g., seasoned mushrooms), 13 salad and vegetable products, 15 deli meats, 4 desserts, 2 egg products, and 4 others. Each sample (approx. 100 g) was homogenized and screened for *L. monocytogenes* following the 3M MDA *Listeria monocytogenes*, and the FDA-BAM methods.

Results: Results from the two detection methods did not differ significantly ($P > 0.05$). Overall, 15/200 samples were positive for *L. monocytogenes* (7.5%). Five samples showed discrepant results; fermented pork and chicken tender sausages tested positive by the FDA-BAM method only. Fermented fish sausage, fried minced mushroom and fried tofu tested positive by the MDA only. The sensitivity of the MDA was 86.7% (95% CI: 58.4-97.7%), specificity was 98.4% (95% CI: 95.0-99.6%), accuracy was 97.5%, and the positive predictive value was 76.9% (95% CI: 46.0-93.8%).

Significance: The 3M MDA *Listeria monocytogenes* yields rapid and reliable results for the detection of *L. monocytogenes* in various food products. Detection and monitoring of *L. monocytogenes* in RTE products are important for the reduction of incidence of listeriosis outbreaks worldwide.

P2-44 Validation of RapidChek F.A.S.T. *Listeria* spp. Test System for the Detection of *Listeria* spp. on Environmental Surfaces

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Introduction: The new USDA-FSIS *Listeria* Control Program requires that ready-to-eat (RTE) food processing facilities test food contact surfaces for *Listeria monocytogenes* or an indicator organism such as *Listeria* spp. In some circumstances, these establishments must withhold the release of product pending *Listeria* test results. In order to reduce the time-to-result and the cost of holding product, we developed a simple, 24-hr, lateral flow test strip-based method for the detection of *Listeria* spp. on environmental surfaces.

Purpose: The purpose of this study was to validate the performance of a new 24-h lateral flow test strip-based method for the detection of *Listeria* spp. on environmental surfaces (stainless steel, plastic, rubber, and painted concrete) under AOAC-Research Institute guidelines.

Methods: For each surface type, 5 non-spiked, 5 high level-spiked, and 20 low level-spiked environmental surfaces were tested by the new lateral flow test strip method at 24 and 40h and the USDA-FSIS cultural reference method. One surface type (stainless steel) was also validated by an Independent Laboratory. Inclusivity/exclusivity studies evaluated 50 *Listeria* strains and 35 non-*Listeria* bacterial strains.

Results: A total of 100 low-level spiked surfaces were tested by both methods. The number of confirmed positives for the RapidChek method tested at 24h and 40h, was 58 and 61, respectively, and 63 for the USDA-FSIS cultural reference method. All non-spiked surfaces were negative for *Listeria* spp. by all methods. The overall Chi-square was 0.520 ($P = 0.471$) and 0.084 ($P = 0.772$) after a 24h and 40h enrichment, respectively. Probability of Detection (POD) analysis showed no significant differences between the test method tested at either 24 or 40h and the USDA cultural reference method. These results indicated that the test method was equivalent in performance to the reference method at both enrichment times. The method showed 100% sensitivity and 100% specificity for *Listeria* spp.

Significance: The new lateral flow test strip-based method should provide industry with a rapid and reliable tool for monitoring and controlling *Listeria* species in the food processing environment and minimize the contamination of food products by *Listeria monocytogenes*.

P2-45 Use of Selected Chemical and Physical Treatments to Reduce *Listeria monocytogenes* Biofilms Formed on Lettuce and Cabbage

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Introduction: Produce is usually consumed raw to benefit human health. However, it also contributes to many outbreaks of *Listeria monocytogenes*, which can form a mature biofilm within 24h. Recent control strategies have proved ineffective in ensuring safe food production.

Purpose: The present study investigated the efficacy of chemical treatment (chlorine, citric, lactic, and malic acid) and physical treatment (ultrasonication, ultraviolet-C, and cold oxygen plasma) on *L. monocytogenes* biofilm on lettuce and cabbage stored at 4°C for 24h.

Methods: The inoculated samples were exposed to 200 ppm chlorine, 2% citric acid, 2% lactic acid, 2% malic acid, 32 Hz ultrasonication for 5 min. Each side of samples was also subjected to 390 mJ/cm^2 of ultraviolet-C (UV-C) and 750 mJ/cm^{2}of cold oxygen plasma (COP).

Results: Two hundred ppm chlorine reduced 0.68 log CFU/lettuce coupon which was statistically ($P < 0.05$) significant different compared to control. Lactic and malic acid also achieved a similar reduction 0.56 log CFU/lettuce coupon whereas citric acid and US did not give a statistically ($P > 0.05$) significant reduction compared to control sample. On the other hand, UV-C and COP significantly reduced ($P < 0.05$) *L. monocytogenes* on lettuce, a reduction of 4.06 and 3.85 log CFU/lettuce coupon was achieved, respectively. However, the chemical treatments seem to be more effective when applied on cabbage. Chlorine with the same concentration eliminated up to 3.35 log CFU/cabbage coupons whereas 2 % lactic and malic achieved a similar reduction (approx. 3.40 log CFU/cabbage coupons). These results were higher than that observed on lettuce. Furthermore, citric acid, which showed no reduction on lettuce, showed a 2.68 log CFU/cabbage coupon reduction effect; while US gained a lower reduction (0.77 log CFU/cabbage

coupon). In addition, *L. monocytogenes* biofilm was reduced with a similar value by UV-C and COP (3.97 and 4.09 log CFU/cabbage coupon, respectively) compared to that on lettuce. The highest reduction for chemical treatment was achieved by chlorine, lactic and malic acid; and the highest reduction for physical treatment was accomplished by the UV-C and COP treatment. However, when compare between the chemical and physical treatment, UV-C and COP gave the best reduction on both lettuce and cabbage.

Significance: This study demonstrates that photosterilization such as UV-C and COP can be an effective mean to overcome the problem of *L. monocytogenes* biofilm on produce.

P2-46 Survivability of Norovirus Surrogate on Various Food-contact Surfaces

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Introduction: Norovirus (NoV) is an environmental threat for humans causing foodborne and waterborne disease and spread easily from one infected person to another. NoV can survive on various environmental surfaces thus it can affect an incident of cross-contamination. However, there is lack of information about the survivability of NoV depending on variety types of food contact surfaces.

Purpose: The objective of this study was to investigate the survivability of NoV using a NoV surrogate, murine norovirus (MNV-1) on six different food-contact surfaces during the 28 days of storage. 10^5PFU of MNV were inoculated on six different coupons and the contaminated coupons were kept for 28 days (4 weeks) at room temperature.

Methods: The prepared coupons (ceramic, rubber, wood, glass, plastic and stainless steel) were soaked in 70% ethanol for 1h and washed with distilled water. We inoculated 10^5 PFU of MNV-1 on six different coupons and kept for 4 weeks (28 days) at room temperature. Each of coupons was taken out at the point of determined times, and 50 µl of elution buffer (0.05 M glycine-0.14 M NaCl buffer (pH 7.5)) was pipetted onto the coupons and left at room temperature for 10 minutes. The coupons were placed into 15 ml conical tubes with 200 µl of elution buffer and vortexed for 10 minutes to elute virus. Then, each eluted viral suspension was 10-fold serially diluted and analyzed by plaque assay. The value of d_R(time required to reduce virus by 90%) was determined by Weibull models.

Results: On the food-contact surfaces, the reduction of MNV was at maximum 2.28 log PFU/coupon on stainless steel, while at minimum 1.29 log PFU/coupon on wood. The highest reduction values were achieved on stainless steel, plastic, rubber, glass, ceramic, and wood, orderly on the virus. The values of D_R (time required to reduce virus by 90%) on the survival plots of MNV determined by modified Weibull model were 277.60 h (R^2=0.99) on ceramic, 492.59 h (R^2=0.98) on wood, 173.56 h on rubber (R^2=0.98), 97.18 h (R^2=0.94) on glass, 91.76 h (R^2=0.97) on stainless steel, and 137.74 h (R^2=0.97) on plastic. The infectivity of MNV on all food-contact surfaces was still active after 28 days.

Significance: This study shows that MNV-1 persists in an infective state on various food-contact surfaces for long periods of time. This study may provide valuable information to control the NoV on various food-contact surfaces for preventing foodborne disease.

P2-47 Influence of NaCl on the Inactivation of Murine Norovirus-1 and Hepatitis A Virus in Korean Traditional Salted Oyster Called "*Eoriguljeot*" during Storage

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Introduction: Enteric noroviruses (NoV) and hepatitis A virus (HAV) are the leading causes of non-bacterial shellfish-borne gastroenteritis. Raw fresh oysters (*Crassostrea gigas*) in Korea are processed into a salted and seasoned traditional food called *Eoriguljeot*. However, it is currently not known whether the addition of NaCl and storage times are sufficient to inactivate NoV and HAV in *Eoriguljeot,* and there is still lack of information and scientific data on the non-thermal inactivation or reduction of specific pathogenic viruses in diverse seafood products (National Advisory Committee on Microbiological Criteria for Foods, 2008).

Purpose: In the present study we examined the effects of various NaCl concentrations on the survival of murine norovirus-1 (MNV-1) as a surrogate of NoV and HAV inoculated in raw oysters to simulate storage conditions for home-made *Eoriguljeot*.

Methods: Fresh oyster of 10 g were infected with 100 µl of the appropriate dilution of each viral stock to obtain the final infection levels of 5.83 log $TCID_{50}$ for MNV-1 and 5.30 log $TCID_{50}$ for HAV. NaCl at 3, 5, 7, or 10% was added to MNV-1 or HAV-contaminated raw oyster samples which were then stored at 10°C for 72h. The 50% tissue culture infectious dose ($TCID_{50}$) method was used to estimate virus titers during the storage. Decimal reduction times at a given concentration of NaCl were also calculated using D = - 1/slope.

Results: Both MNV-1 and HAV titers were significantly ($P < 0.05$) decreased by stepwise increase in NaCl and storage time; however, MNV-1 was more resistant to NaCl than HAV. The overall reductions in MNV-1 and HAV titers in oysters incubated with 5%, 3%, and 0% NaCl were 0.61 and 1.20, 0.50 and 0.89, 0.35 and 0.64, 0.25 and 0.42, and 0.07 and 0.25 log $TCID_{50}$ (50% tissue culture infectious dose)/ml, respectively. The 1.23 log (94.11%) reduction of MNV-1 and 2.45 log (99.65%) reduction of HAV survival also occurred in 10% NaCl-containing oysters by 72h of storage. Using the first-order reaction model we determined that in 10% NaCl the MNV-1 decimal reduction time (D-value) was 64h, whereas for HAV D-values of 63h, 47h, and 31h were achieved in 5%, 7%, and 10% NaCl, respectively.

Significance: This study suggests that NaCl in concentrations over 10% could be potentially used in conventional *Eoriguljeot* as an antiviral agent because HAV was almost completely inactivated (99%) by 10% NaCl.

P2-48 Growth Potential of *Listeria monocytogenes* in Artificially Contaminated Celery and Chicken Salad

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Introduction: In a recent outbreak of listeriosis, celery used to make chicken salad was identified as the vehicle for contamination. This outbreak raised questions about *Listeria monocytogenes* (Lm) growth in these foods and the implications for infective dose.

Purpose: The purpose of this study is to assess the ability of Lm, including the outbreak strains, to grow in the mayonnaise-based chicken salad and in celery.

Methods: Chicken salads and celery were purchased at retail or prepared using the recipe involved in the outbreak. An antibiotic-resistant cocktail of 1/2a, 1/2b and 4b Lm strains were used to inoculate celery and chicken salad at 3-6 x 10^3 CFU per 5 grams. The inoculated samples were stored at 5°, 10° and 25°C. Samples were withdrawn at different intervals and plated onto BHI agar containing selective antibiotics and incubated at 37°C for 24-48 hours before colonies were counted.

Results: The growth of all three serotypes in celery and in both retail and lab-made chicken salads were comparable. Lm grew slowly at 5°C. Final counts reached 8.0 x 10^7 /5g at 5°C after 30 days of incubation. The doubling time varied from 30 to 60h at 5°C, 36 – 48h at 10°C and 1.3 – 10.6h at

25°C. The salads had a water activity of 0.99 and average pH ranged from 5.9 to 5.3. Growth in diced celery reached 10^4-10^5 CFU/5g at 5°C, after 20 days.

Significance: These data show that celery and chicken salad, retail as well as homemade, can support Lm growth. The outbreak investigation found that the implicated salad was properly refrigerated and stored for only a few days, indicating limited growth of Lm in the implicated outbreak salad. This means that the infective dose was almost entirely due to the number of Lm cells contributed by the contaminated celery.

P2-49 Ultraviolet-C Light Inactivation of *Listeria monocytogenes* on Organic Fruit Surfaces

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Introduction: Ultraviolet-C (UV-C) light treatment can effectively be utilized for disinfecting food surfaces.

Purpose: This study examined the effect of UV-C dose and fruit surface characteristics on the inactivation kinetics of *Listeria monocytogenes* on the surface of organic apple, pear, strawberry and cantaloupe.

Methods: Fruit surfaces spot inoculated with a cocktail of *L. monocytogenes* (NRRL B-33006; NRRL B-33069 and NRRL B-33385) were exposed to UV-C light (0 to 11.9 ± 0.32 KJ/m^2) at 23°C. Weibull model was used to describe the inactivation kinetics.

Results: *L. monocytogenes* reduction was higher on apples (1.61 log CFU at 3.75KJ/m^2) and pears (1.54 log CFU at 11.9KJ/m^2) surface compare to cantaloupes (0.95 log CFU at 11.9KJ/m^2) and strawberries surface (0.95 log CFU at 11.9KJ/m^2). For all fruits, surface reduction achieved within the first minute of treatment (1.10 ± 0.16 KJ/m^2) were significantly ($P < 0.05$) higher as compared to the remaining 2 to 13 min of the treatment. The shape parameter (p) obtained from Weibull model were between 0.12-0.28 for all fruits indicating that the remaining microbes were resistant to inactivation or had the ability to adapt to the applied stress. The time for first decimal reduction was lower in apples (63 s) and pears surface (141 s) compare to cantaloupes (1320 s) and strawberries (3025 s) surface. The root mean square surface roughness as observed from a Stylus surface profilometer was higher on strawberry (296 µm) and cantaloupe (55.8 µm) than apple (30.3 µm) and pear (40.2 µm). The rough surface of strawberry and cantaloupe may have shadowed the microbial cells, impairing the germicidal effect of UV-C light.

Significance: UV-C light is effective in reducing *L. monocytogenes* from fruit surfaces but its efficacy is influenced by their surface characteristics.

P2-50 Cold Shock Protein Functions Contribute to Listeriolysin O Production and Antibiotic Stress Tolerance in *Listeria monocytogenes*

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Introduction: Cold shock proteins (Csps) are small multifunctional nucleic acid binding proteins used to regulate various gene expression and stress adaptation responses in bacteria. *Listeria monocytogenes* is a gram-positive foodborne pathogenic bacterium causing serious illness (listeriosis) and high mortality among those with diminished immunity. This bacterium harbours three Csps whose contribution to virulence and stress resistance responses is not yet fully investigated.

Purpose: Our aims in the present study were to investigate the contribution of the Csps to the regulation Listeriolysin O (LLO) production and antibiotic stress resistance in *L. monocytogenes*.

Methods: *L. monocytogenes* EGDe and its isogenic triple deletion mutant (EGDe Δ*cspABD*) lacking *csp* genes (*cspA*, *cspB* and *cspD*) were compared in terms of LLO expression and sensitivity to different antibiotics.

Results: The EGDe Δ*cspABD* mutant was impaired in hemolysis and LLO production compared to the parental EGDe strain. In addition this mutant was more sensitive than the parental strain to various antibiotics including those used to treat listeriosis (ampicillin and gentamicin). The decreased LLO synthesis in EGDe Δ*cspABD* was associated with reduced expression and stability of *hly* mRNA. Deletion of *csp* genes was not associated with decreased expression of selected multidrug resistance (MDR) transporter genes examined. The EGDe Δ*cspABD* mutant however displayed differences in the expression of genes encoding putative peptidoglycan synthesis proteins (*lmo1438*, *lmo2754*, *lmo2039*, *lmo0540* and *lmo1892*) compared to the parental strain suggesting that loss of Csp functions might have caused alterations to the composition and integrity of the cell wall in this mutant.

Significance: Our results thus indicate that, besides documented roles in cold and osmotic stress adaptation, the *csp* gene family also contributes to the regulation of virulence and antibiotic stress responses in *L. monocytogenes*.

P2-51 Desiccation of *Listeria monocytogenes* Biofilms Reveals an Association between Survival and the Presence of Clonal Complex 8, *Listeria* Genomic Island 1, or Stress Survival Islet Gene Markers, in Outbreak and Environmental Strains

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* is a pathogenic foodborne microorganism noted for its ability to persist for long periods in the environment and food processing facilities. Persistence has been attributed to the bacterium's ability to form biofilms but may also be related to desiccation resistance.

Purpose: The purpose of this study was to characterize the desiccation survival in biofilms formed by environmental and outbreak associated strains of *L. monocytogenes*.

Methods: Pre-formed biofilms of 15 outbreak and environmental *Listeria monocytogenes* strains (serotype 1/2) were desiccated on stainless steel coupons at ~48% relative humidity at 15°C. Viable counts were determined over the course of 21 days. Presence of genetic markers indicative of strains harbouring the stress survival islet (SSI-1), the 50 kbp *Listeria* genomic island (LGI1), and clonal complex 8, virulence type 59 (CC8/VT59) associated with previous Canadian outbreaks, were determined by PCR.

Results: The most desiccation susceptible strains (>2-3 log CFU/cm^2 reduction at 21 days) contained none of the SSI-1, LGI1, or CC8/VT59 markers. In contrast, the majority of strains exhibiting desiccation resistance by undergoing less than a 2-log CFU/cm^2 reduction over the desiccation period of 21 days were observed to harbour at least one of the CC8/VT59, LGI1, and/or SSI-1 markers. Notably, the environmental origin (aquatic, food and human outbreak cases) of *L. monocytogenes* strains appeared to be unrelated to desiccation tolerance as one of the most resistant strains originated in a watershed, while some susceptible strains were isolated in food processing facilities or had caused foodborne illness.

Significance: This study revealed the possible involvement of genes previously associated with environmental persistence in the desiccation survival of *Listeria monocytogenes* biofilms on food grade stainless steel surfaces.

Poster

P2-52 Unraveling the Correlation between Survival, Growth and Transcriptional Boundaries of *Listeria monocytogenes* Following Habituation to Sublethal Acid and Osmotic Stress

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* is capable of elucidating adaptive response to adverse conditions, which may harden the organism against lethal stresses. Nevertheless, transcriptional changes underpinning stress responses close to conditions marginal for growth are poorly evaluated.

Purpose: The study aims to correlate the changes in expression of stress- and virulence-associated genes of *L. monocytogenes* following habituation under suboptimal pH and NaCl, with the survival under extreme acid stress.

Methods: Tryptic Soy Broth, supplemented with 0.6% Yeast Extract (TSBYE) with various combinations of pH (4.6-6.4) and NaCl (2-10% w/v) was prepared in triplicate, inoculated with two *L. monocytogenes* strains (C_5, 6179) separately and stored at 7°C for thirteen days. Growth followed by survival (log reductions, $D_{pH:2.0}$-values) against severe acid stress (TSBYE, pH 2.0 adjusted with HCl) were assessed on day 2, 4, 6, 8, 10 and 13. Relative transcription of *gad2, sigB* and *prfA*, compared to control (pH 7.2, day 0) were estimated with quantitative RT-PCR.

Results: Inter-strain variation in log changes, regarding growth, was evident at pH<5.5 and NaCl>8%w/v, manifested by C_5 exhibiting higher survival (2-3 log reduction) than 6179, which reduced by 4-6 log CFU/ml by the end of storage. During storage, growth/no growth interface for C_5 was slightly shifted to higher NaCl and lower pH values than the respective interface of 6179, while for both strains, survival/death interfaces post acid challenge were similar ($P > 0.05$) throughout storage. Principal Component Analysis pointed out that high relative transcription levels of *gad2* (Fold Changes>30) and *sigB* upregulation (FC>1) correlate with increased D-values (biphasic inactivation curve, D_1:0.8-5min) and acid resistance (2-4 log reductions). Noteworthy, induction of *prfA* prior or post acid challenge reveals the underlying potential risk resulting from exposure to suboptimal or lethal conditions.

Significance: Correlating transcriptional response of *L. monocytogenes* with growth/no growth or survival/death interface, results in stochastic description of stress resistance and virulence. Such a description is of high value for designing safe intervention strategies. It also assists in better understanding the impact of processing on bacterial physiology and integrates –omics in quantitative microbial risk assessment. This work has been supported by the project "Efficacy of NOVEL analytical techniques to prEdict the quality and safetY of newly developed pErishable food products 11SYN_2_1528" co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the O.P. "Competitiveness and Entrepreneurship (OPC II)" ROP Macedonia – Thrace, ROP Crete and Aegean Islands, ROP Thessaly – Mainland Greece – Epirus, ROP Attica, Framework NSRF 2007-2013, COOPERATION 2011.

Poster

P2-53 Growth Differences of Two Strains of *Listeria monocytogenes* in Defined Medium Using Glucose as the Sole Carbon Source

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Introduction: The foodborne pathogen *Listeria monocytogenes* is capable of surviving in diverse and stressful ecological habitats and work in the past 30 years has identified *L. monocytogenes* serovars which are predominantly responsible for outbreaks. As has been shown with rumen and other microbial ecologies, differing affinity responses to carbon and nitrogen sources have helped explain why certain species dominate in particular ecologies.

Purpose: This study's purpose was to investigate strain-to-strain growth variation in growth *of L. monocytogenes* on glucose.

Methods: *L. monocytogenes* strains Scott A and EGD-e were grown in TSB and then in a defined medium (DM) with 10 mM glucose. Late exponential phase cultures in DM were used as inoculum for both tubes and 96-well plates which were incubated at 37°C. Glucose concentrations ranged from 100 mM to 62.5 µM for 96-well plates and 13.8 mM, 55.5 µM, and 555 nM for tubes. Counts of colony forming units (CFU) on tryptic soy agar from tubes were used to calculate growth kinetics.

Results: Plate CFU counts from 3 biological replicates demonstrated differences in growth rates between EGD-e and Scott A for 555 nM only (0.26 ± 0.02 h^{-1} versus 0.18 ± 0.01 h^{-1}; $P < 0.05$). Both strains exhibited stationary phase for 13.8 mM but transitioned quickly into death phase at 55.5 µM. In 96-well plates, EGD-e in glucose above 5 mM demonstrated traditional exponential growth and transition into stationary phase. Whereas, Scott A also demonstrated rapid growth and death without a discernable stationary phase. The slope of death often shouldered with two stages, the second steeper than the first.

Significance: These data suggest that growth rates of *L. monocytogenes* are affected by both strain and glucose concentration. Thus some strains may influence the outgrowth of others, potentially accounting for different recovery frequencies from different environments.

P2-54 Virulence-attenuated *Listeria monocytogenes* Strains Raise T Cell-mediated Immunity and Confer Protection against a Subsequent Challenge by Fully-virulent *L. monocytogenes* Strains

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Introduction: *L. monocytogenes* (*Lm*) isolates from food commonly (45%) carry virulence-attenuating mutations in *inlA*. Individuals are frequently exposed to foodborne *Lm* without developing disease, and natural exposure to virulence-attenuated (VA) strains could contribute to maintained population immunity against listeriosis.

Purpose: Mouse challenge experiments were conducted with murinized *Lm* strains to determine if VA *Lm* strains are capable of (i) eliciting a *Lm*-specific memory $CD8^+$T cell response, and (ii) providing protection against a subsequent challenge with a fully-virulent strain.

Methods: Mice were orally administered doses of a VA *Lm* strain, a fully-virulent *Lm* strain, or *L. innocua* as a "vaccination," or a PBS placebo ("unvaccinated"). Forty days post-inoculation animals were either (i) sacrificed and $CD8^+$ T cells were isolated from spleens and examined for INF-g production and memory phenotyping by flow cytometry using anti-CD44 and -CD62L antibodies, or (ii) challenged with a fully-virulent strain at $10^7$to probe protective immunity through exposure to VA strains as measured by bacterial counts from livers, spleens, and small intestines.

Results: Memory cell phenotype and $CD8^+$ T cell INF-g frequency (~1.6%) were similar for VA and fully-virulent strains at 10^7. Mice vaccinated with the VA strain at 10^5 CFU showed reduced bacterial loads in internal organs, indicating reduced severity of infections in mice exposed to VA strains. Specifically, in unvaccinated animals 2.52 and 1.63 mean log CFU of the challenge strain was recovered in livers and small intestines, respectively, as compared to 1.94 and 1.16 mean log CFU in organs for vaccinated animals. Interestingly, mice vaccinated with the fully-virulent strain at 10^3CFU did not show a protective effect, as log CFU in organs were similar in vaccinated vs. unvaccinated groups.

Significance: Data support that VA *Lm* strains commonly in food elicit a memory $CD8^+$ T cell population leading to long-lived protection. Data on VA *Lm* immune responses will be communicated to food safety policy makers, as they correlate to levels of predicted human exposure through consumption of contaminated foods.

P2-55 Insertional Mutagenesis Reveals Genes That May Contribute to *Listeria monocytogenes*' Desiccation Tolerance

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Introduction: The foodborne pathogen *L. monocytogenes* can survive desiccation for extended periods (> three months) in a simulated food processing environment. However, the mechanisms used by *L. monocytogenes* to remain viable in the low relative humidity (RH) environment on the surface of food processing equipment are largely understudied.

Purpose: The objective of this study was to investigate molecular mechanisms which may contribute to desiccation tolerance in *L. monocytogenes*.

Methods: A library of 11,700 *Himar1 L. monocytogenes* 568 transposon insertion mutants was initially screened in a microplate assay for strains displaying increased or decreased survival when desiccated in TSB at 43% RH and 15°C. The desiccation phenotypes were subsequently assessed on food grade stainless steel coupons. Sequencing of arbitrary PCR products was used to determine transposon insertion sites in strains exhibiting > 0.5 log CFU/cm^2 change in survival after seven days of desiccation on the stainless steel coupons. Strain morphology and survival in TSB+20% NaCl was also analyzed.

Results: Initial screening led to the selection of 129 sensitive and 61 tolerant desiccation mutants. Further testing on stainless steel yielded 15 mutants each with increased or decreased desiccation survival compared to the wildtype. Of the 15 sequenced desiccation tolerant mutants, 7 immotile mutants contained *Himar1*inserts in motility related genes while the remaining mutants harboured inserts in genes involved in fatty acid metabolism, membrane transport, protein biosynthesis, transcription regulation, and virulence. Interrupted genes in the 15 sensitive mutants encode a variety of functions including lipid membrane and protein synthesis, transport, surface and stress proteins. A significant (P = 0.01) positive correlation existed between the survival under desiccation and osmotic stresses, demonstrating cross-protective mechanisms between the two types of water stress.

Significance: This study revealed genes which may contribute to the survival and persistence of *L. monocytogenes* exposed to low RH conditions in the food processing environment.

P2-56 Analysis of the Transcriptomes Related to the NaCl Effect on *Listeria monocytogenes* Resistance and Its Pathogenicity

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Developing Scientist Competition

Introduction: NaCl has been used in processed meat products to improve flavor and food safety because NaCl may sensitize foodborne pathogens to subsequent stresses. However, some of *L. monocytogenes* strains may not be sensitized by NaCl, and its pathogenicity was also increased by NaCl.

Purpose: The objective of this study was to identify the genes activated by NaCl, which prevents *L. monocytogenes* from sensitizing against heat stress and increases the pathogenicity.

Methods: Nine strains of *L. monocytogenes* were inoculated individually in 10 ml tryptic soy broth plus 0.6% yeast extract, which was supplemented with 0, 1, 2, and 4% NaCl to be exposed to NaCl, and the cells were then subjected to at 60°C for 60 min to select *L. monocytogenes* strains, which are not heat-sensitized by NaCl. After heat challenge, *L. monocytogenes* strains were categorized into heat-sensitized strains (HS) and non-sensitized strains (NS). mRNA was then extracted from HS and NS, and cDNA was prepared to analyze transcriptomes (*inlA*, *inlB*, *opuC*, *ctc*, *betL*, *osmC*, and *gbuB*) by qRT-PCR.

Results: After heat challenge *L. monocytogenes* strains were categorized in to HS (*L. monocytogenes* strains NCCP 10805, NCCP 10806, NCCP 10807, NCCP 10810, NCCP 10811, and NCCP 10920) and NS (*L. monocytogenes* strains NCCP 10808, NCCP 10809, and NCCP 10943). Of seven tested genes, gene expression levels of pathogenicity related genes (*inlA* and *inlB*) and stress response genes (*opuC*, *betL*, and *ctc*) increased (P < 0.05) for NS (*L. monocytogenes* strains NCCP 10808 and NCCP 10809) after being exposed to NaCl as NaCl concentration increased. However, only *betL* gene expression level for HS (*L. monocytogenes* NCCP 10811) was increased (P < 0.05) as NaCl concentration increased.

Significance: The effect of NaCl on heat-sensitizing *L. monocytogenes* is strain-dependent, and *opuC* and *ctc* genes may prevent NS from heat-sensitizating by NaCl. Moreover, NaCl also increases invasion-related genes (*inlA* and *inlB*).

P2-57 *Listeria monocytogenes*, *Listeria welshimeri*, *Listeria seeligeri* Inter-species Competition in Buffered *Listeria* Enrichment Broth

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Introduction: Because the levels of contamination with *L. monocytogenes* are frequently less than 1 CFU/g, selective enrichment is typically necessary for isolation. Current selective enrichment formulations are only genus specific, thus the presence of additional *Listeria* species may hinder subsequent *L. monocytogenes* recovery particularly on non-species differentiating media such as Oxford or PALCAM agar.

Purpose: The purpose of this study was to evaluate the effects of *L. welshimeri* and *L. seeligeri* on subsequent isolation of *L. monocytogenes* from buffered *Listeria* enrichment broth (BLEB).

Methods: 2% UHT milk was doubly spiked with *L. monocytogenes* and *L. welshimeri* (or *L. seeligeri*). Selective enrichments using BLEB were performed. Population differentials at 48h were determined based on qPCR enumeration. Select strain pairings were then used for additional spike-recovery studies in four distinct food matrices using Oxford agar as the recovery medium with confirmation of 10 colonies per enrichment.

Results: Population differentials as much as 2.7 ± 0.1 and 3.7 ± 0.2 logs were observed for *L. monocytogenes* paired with *L. seeligeri* and *L. welshimeri*, respectively. In all pairings, *L. monocytogenes* was the less predominant species. Population differentials of 2.7 and 3.7 logs would require confirmation of approximately 500 and 5000 colonies respectively for *L. monocytogenes* recovery. The presence of *L. seeligeri* had minimal effects while *L. welshimeri* completely masked the presence of *L. monocytogenes* in spiked guacamole, crab meat, and broccoli. No clear relationship between the predominant species and generation time or ability to produce an inhibitory activity was observed.

Significance: The presence of *L. welshimeri* or *L. seeligeri* during selective enrichment using BLEB can hinder recovery of *L. monocytogenes* on non-differentiating media such as Oxford agar. Additionally, the large population differentials suggest that in some cases isolation of *L. monocytogenes* would be difficult even using a species-specific chromogenic agar.

P2-58 Effect of Humidity and Storage Temperature on the Survival of *Salmonella* in Paprika and Black Pepper

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Introduction: *Salmonella* is an ongoing concern as a vehicle for foodborne outbreaks in low-moisture foods such as dried spices. It has been shown to persist for years during storage of spices. However, recent publications indicate a dramatic effect of humidity and temperature on the survival of *Salmonella* during storage. The exact relationship between temperature and humidity and their influence on the survival of *Salmonella* during spice storage has not been carefully examined.

Purpose: The objective of this study is to determine how prolonged storage at various temperatures and humidity levels affects the persistence of *Salmonella* in spice using paprika and ground black pepper as model spices.

Methods: A cocktail of four different *Salmonella* serovars was inoculated at approximately 7-8 log CFU/g into 1 g samples of paprika or ground black pepper. The samples were stored at different temperature (10, 25 and 37°C) and humidity (32, 43, 57 and 80 %) combinations for up to 2 years. Survival was determined by a brief enrichment in tryptic soy broth with yeast extract followed by enumeration on both tryptic soy agar with yeast extract and xylose lysine desoxycholate agar.

Results: Storage at 37°C resulted in a loss of detection of *Salmonella* at a more rapid rate when compared to lower temperatures. As the humidity was increased at 37°C, *Salmonella* survival decreased from ~30 days (32% humidity) to only a matter of hours (80% humidity). However, at the lower temperatures, *Salmonella* continued to persist with no remarkable decline in population even when combined with high humidity. Statistically, a correlation between type of spice, storage temperature, relative humidity and survivability was established.

Significance: Results of this study indicate that storage temperature plays a greater role in the persistence of *Salmonella* in these low-moisture foods as compared to the humidity.

P2-59 Efficacy of Peroxyacetic Acid Wash against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* on Citrus Fruits

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Poster

Introduction: All fruit and vegetable juices sold as juice or for use as an ingredient in other beverages are subject to the Juice HACCP regulation. Plant HACCP programs identify the washing process as part of the kill step for citrus juice products. Citrus juice processors may apply single or multiple treatments to fruit surfaces, provided that the 5-log reduction process begins after culling and cleaning, and the reduction is accomplished within a single production facility.

Purpose: The objective of this study was to determine the antimicrobial efficacy of peroxyacetic acid (PAA) wash against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* on citrus fruits.

Methods: Oranges, lemons, limes and grapefruits were surface inoculated with *E. coli* O157:H7, *L. monocytogenes* and *Salmonella.* Inoculated samples were washed with 13.6 ppm or 80 ppm PAA for 5 min.

Results: Variations among the citrus fruits and test pathogens were observed. The 13.6 ppm PAA treatment for 5 min was effective in reducing counts of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on oranges *by* 4.75 log, 3.96 log and 5.03 log, respectively. The populations of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* were reduced, on average, by 3.69 log, 4.44 log and 3.81 log, respectively, on lemons, limes and grapefruits when treated with the 80 ppm solution of PAA for 5 min.

Significance: The experimental test results showed that the 80 ppm PAA treatment for 5 min in combination with an additional surface treatment that delivers 1.5 to 2-log reduction of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on oranges, lemons, limes and grapefruits will achieve the 5-log pathogen reduction performance standard. Alternatively, the 80 ppm PAA treatment for longer than 5 min may deliver the required 5-log reduction on citrus fruits.

P2-60 Developing a Two-step Heat Treatment for Inactivating Desiccation-adapted *Salmonella* in Aged Chicken Litter

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Developing Scientist Competition

Introduction: Chicken litter may contain a variety of human pathogens, such as *Salmonella*, that can potentially contaminate fresh produce as an organic fertilizer. Some bacterial cells become acclimatized to desiccation condition in stockpiled chicken litter and develop cross-protection to subsequent dry-heat processing. Dry-heat treatment alone may not readily decrease the desiccation-adapted pathogenic cells to safe levels, resulting in the survival of some heat-resistant desiccation-adapted cells.

Purpose: The objective of this study was to evaluate the effectiveness of a two-step heat treatment for aged chicken litter on elimination of desiccation-adapted *Salmonella*.

Methods: Aged chicken litter with 20, 30, 40, and 50% moisture contents was inoculated with a mixture of four *Salmonella* serotypes for a 24-h desiccation adaptation. Afterwards, the chicken litter with desiccation-adapted cells was added into litter with the same moisture contents for a 1-h wet-heat treatment at 65°C and 100% RH inside a water bath. The inoculated litter was then dry-heated in a convectional oven at 85°C for 1h to the desired moisture level (<12%).

Results: After wet-heat treatment, the populations of *Salmonella* in aged chicken litter at 20 and 30% moisture contents decreased from 6.7 log CFU/g to 3.3 and 3.0 log CFU/g, respectively, and after subsequent dry-heat treatment, the populations decreased to 3.0 and 2.6 log CFU/g, respectively. *Salmonella* cells in litter samples at 40 and 50% moisture contents were only detectable by enrichment for 40 and 20 min of wet-heat treatment, respectively. Moisture contents in all samples were reduced to <12% after drying process.

Significance: Our results demonstrated that the two-step heat treatment was effective in reducing >5.5 logs of desiccation-adapted *Salmonella* in chicken litter with moisture content at or above 40%. Therefore, the findings from this study provide the industry with a cost-effective heat treatment method for processing chicken litter.

P2-61 Competitive Interactions Inside Mixed-culture Biofilms of *Salmonella* Typhimurium and Cultivable Indigenous Microorganisms on Lettuce Enhance Microbial Resistance of Their Sessile Cells to Ultraviolet C (UV-C) Irradiation

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Introduction: *Salmonella* Typhimurium (ST) is one of the leading causes of foodborne diseases in fresh produce, such as lettuce. Despite this, the role of the possible interactions between lettuce indigenous microorganisms and ST on their ability to form biofilm on lettuce and subsequently on the sensitivity of their sessile cells to ultraviolet C (UV-C) irradiation, remains relatively unexplored.

Purpose: Here, the interaction of a mixed-culture of ST and cultivable indigenous microorganisms (CIMs) was examined, as well as the efficacy of UV-C.

Methods: Initially, the CIMs were isolated and cultured with ST at 15°C either planktonically or left to form biofilms on stainless steel (SS) and lettuce leaves. Microbial growth, biofilms formation, and survival following UV-C treatment were monitored using traditional plate count methods while biofilm formation, production of extracellular polymeric substance (EPS), and stomatal colonization were also observed by field emission scanning electron microscopy (FESEM). Internalization strength, color, and texture were analyzed by standard methods.

Results: It was revealed that the mixed-culture of ST and CIMs presented significantly (P <0.05) decreased biofilms formation on lettuce leaves compared to mono-cultures (i.e., ST or CIMs alone), which indicated competitive interaction between them, while no interactions were observed for biofilms on SS and for the planktonic cultures. It was also demonstrated that a mixed-culture biofilm on lettuce presented significantly higher resistance (P < 0.05) to UV-C treatment compared to mono-cultures biofilms, but such an effect was not observed for biofilms formed on SS and for the planktonic cultures. The Weibull model fitted well to microbial inactivation curves with R^2 values that ranged from 0.90 to 0.97. Regarding the mixed-culture conditions, a UV-C fluency of 35 mJ/cm^2 was required to achieve a 5.0 log CFU/ml or cm^2 reduction in planktonic and biofilms on the SS for the mixed-culture, while 360 mJ/cm^2 was required to reduce biofilm cell number by approximately 2.0 log CFU/cm^2 on lettuce. Furthermore, FESEM analysis indicated higher EPS production, and greater stomatal colonization on lettuce mixed-cultures compared to mono-cultures. Finally, internalization strength was significantly higher (P < 0.05) for the mixed-culture on lettuce, thus supporting the notion that internalization in lettuce is a factor contributing to microbial UV-C resistance. The UV-C doses (360 mJ/cm^2) did not change the color and texture of lettuce leaves.

Significance: Therefore, UV-C could be an alternative to chlorine disinfectants for lettuce leaves, even high efficacy is required as lettuce possess indigenous microorganisms.

P2-62 Diversity of the CRISPR-cas system in *Salmonella* Bareilly

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Developing Scientist Competition

Introduction: *Salmonella enterica* subspecies *enterica* serotype Bareilly (*S.* Bareilly) is one of the top 20 *Salmonella* serotypes causing human disease in the US. Studies on the diversity of the Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-*cas* system in *S.* Bareilly are not yet available.

Purpose: To explore the use of the CRISPR-cas system to discriminate between *S.* Bareilly isolates, and to study the association between epidemiological characteristics and CRISPR in *S.* Bareilly.

Methods: Because the genome assemblies often break inside the CRISPR array, this region was amplified and Sanger sequenced for 50 *S.* Bareilly strains, comprising different PFGE patterns. Two new primers were designed for this experiment. Repeats were extracted using CRISPRdb, and the individual spacers and array organization were compared and described across strains. Phylogenetic trees (4) were reconstructed from concatenated genes mined from WGS data: *cas* genes, housekeeping genes (MLST), and leader sequences 1 and 2. Additionally, strains were tested for antimicrobial susceptibility.

Results: *S.* Bareilly displayed 1 type of CRISPR1 and 2 types of divergent CRISPR2 arrays. Leader1 sequences were conserved across strains, but phylogenetic trees of *cas* genes, housekeeping genes, and leader2 sequences grouped strains in 2 clusters, similarly to CRISPR2. Overall, strains with similar CRISPR2 arrays also clustered together for the other genomic elements (*cas* genes, leader2 sequence and MLST genes). Clustering by CRISPR array content or phylogenetic trees did not correlate with geographical origin of the isolate or food vehicle. All strains displayed susceptibility to every antimicrobial tested, and a correlation between antimicrobial resistance and CRISPR elements was not established.

Significance: Although CRISPR arrays could differentiate strains in two groups, they lacked the discriminatory power suitable for outbreak investigations.

P2-63 Bioinformatics Analysis of *Salmonella fliC* Gene Diversity from Next-generation Sequencing Data

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Introduction: Standard *Salmonella* serotyping methods rely on the detection of somatic (O) and flagellar (H) antigens present on the cell surface. *Salmonella fliC* gene, encoding *Salmonella* phase 1 H antigen, is one of the *Salmonella* serotype determinant genes. Next-generation sequencing (NGS) technology has recently been widely applied in clinical and public health laboratory investigations for pathogen detection and surveillance. Hundreds of *Salmonella* strains had been collected from food, clinical and environmental sources and their whole genome sequences were obtained by NGS technology.

Purpose: The purpose of this study was to investigate the genetic diversity of *Salmonella fliC* gene to discover biomarkers for rapid serotype detection.

Methods: A bioinformatics pipeline was developed and implemented for sequence acquisition and genetic diversity analysis from NGS data. It consisted of several steps: reference sequence retrieval and template sequence determination; retrieval of NGS sequence reads of *Salmonella* outbreak isolates; multiple sequence alignment and phylogenetic analysis.

Results: The *flic* reference sequences of 24 *Salmonella* strains of 13 serotypes were retrieved from National Center for Biotechnology Information (NCBI) database, and the phylogenetic tree revealed the relationships among the 13 serotypes based on SNPs variations in the data set. The genetic diversity of *Salmonella fliC* gene was distinguished by applying the pipeline on the NGS reads of 48 *S*. Newport, 48 *S*. Montevideo, and 115 *S*. Enteritidis outbreak isolates, respectively. The marker sequences for *Salmonella fliC* gene were identified.

Significance: The developed pipeline provides an effective bioinformatics tool for genetic diversity clarification and marker sequences discovery which will enhance the NGS data analysis and its applications on pathogen identification, source tracking, and population genome evolution.

P2-64 Development of *Salmonella* Heidelberg Mutant Library Using a Transposon Mutagenesis System

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Developing Scientist Competition

Introduction: *Salmonella enterica* with 1500 serovars has been considered as one of the most prominent foodborne pathogens in poultry industry. In last several years, the incidence of *Salmonella* Heidelberg was increased significantly in the USA and identified as one of the primary serovars associated with human illness. Although *S*. Heidelberg exhibits similar antigenic characteristics with *S*. Typhimurium one of the more common serovars in human diseases, *S*. Heidelberg has not been investigated nearly to the same extent.

Purpose: The aims of this research were to develop *S*. Heidelberg mutant library using transposon mutagenesis and identify essential genes for survival and virulence that could be used a potential targets for more effective intervention methods.

Methods: *Salmonella* Heidelberg ARI-14 isolated from poultry was evaluated for ampicillin and kanamycin susceptibility prior to transposon mutant library construction because the EZ-Tn5 pMOD-6 plasmid DNA possesses both antibiotics genes as selection markers. The EZ-Tn5 pMOD-6 plasmid DNA was transformed to *S*. Heidelberg strain in advance to improve efficiency through host adaptation followed by development of a transposon mutant library.

Results: *Salmonella* Heidelberg ARI-14 strain was susceptible ampicillin (100 µg/ml) and kanamycin (60 µg/ml). The efficiency of EZ-Tn5 pMOD-6 plasmid DNA transformation to ARI-14 for host adaptation was 10^6cells per ml. The excised transposon from host-adapted plasmid DNA was utilized for mutant library.

Significance: The developed *S*. Heidelberg mutant library can be applied to various growth conditions like low pH and thermal stress to screen essential genes for survival and virulence. Identification of these genes will help to elucidate more effective control measures in food systems.

P2-65 Bile Affects Expression of Virulence and Iron Acquisition Genes in Foodborne STEC and *Salmonella enterica* Pathogen Isolates

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Poster

Introduction: Foodborne pathogens *Escherichia coli* (STEC) and *Salmonella enterica* are responsible for large-scale recalls of fresh produce, retail meat, and nuts. These pathogens respond to their environment (food and inside the host) by changes in gene expression under nutrient limited growth conditions.

Purpose: The objective of this study was to assess the transcriptome of important foodborne enteric pathogens, especially virulence gene expression in response to bile, mimicking stress conditions in the host intestinal environment.

Methods: Outbreak associated isolates of *E. coli* O157:H7, *E. coli* O104:H4 and *Salmonella enterica* serovar Montevideo were selected. *E. coli* MG1655 was used as a non-pathogenic control. Cells were grown in DMEM, with and without complex bile acids and short chain fatty acids to simulate the intestinal environment. RNA was harvested at mid-log phase and hybridized to custom high density gene expression (FDA-ECSG and FDA-SEEC, respectively, for *E. coli* and *Salmonella*) microarrays. The arrays contain probe sets derived from chromosomal and plasmid sequences representing the pan-genomes of these pathogens.

Results: Bile supplemented media triggers expression of iron stress response, oxidative stress response and membrane efflux in pathogens, indicating a survival mechanism in an iron-scarce environment similar to previous reports. Additionally, our data show that bile suppresses expression of major virulence factors by at least two-fold, including genes for adherence, type three and four secretion system proteins, Shiga toxins (*stx*2 in *E. coli* O157:H7), and those found in pathogenicity islands.

Significance: The findings indicate that some foodborne enteric pathogens react to bile through wide-ranging changes in gene transcription. We identify genes that help pathogens adapt to the changing environmental conditions and host defenses encountered during human infection. Comparing these genes expressed in the host to those expressed on food as reported by others, will help identify functional genomic key stress response markers that can be used for rapid detection of pathogens on food.

P2-66 Performance Evaluation of DuPont™ BAX® System Real-time PCR Assay for *Salmonella* Enteritidis and Typhimurium

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Introduction: *Salmonella* Enteritidis and Typhimurium are among the top serovars significant in public health. In response to demands for a faster, simpler method for identifying these organisms, A novel Assay was developed for *Salmonella* Enteritidis and Typhimurium (including its monophasic variant, 4,5,12:i:-).

Purpose: To evaluate several key performance characters of this assay, including inclusivity, exclusivity, limited of detection (LOD), and food and environmental testing.

Methods: Inclusivity was evaluated using 165 strains of Enteritidis and 335 strains of Typhimurium isolated from diverse geographic locations and sources, and 524 strains of non-target *Salmonella* (representing 166 serovars) and 30 non-*Salmonella* strains were used for exclusivity. For LOD, ground beef enrichments were artificially spiked with known concentrations of *Salmonella* and serially diluted for testing. For food and environmental testing, pre- or post-enrichment spiked samples of chicken breast, chicken rinse and chicken house swabs were used.

Results: The assay demonstrated >99.4% inclusivity and 100% exclusivity. LOD was estimated at ≤10^4 CFU/ml after enrichment. The sensitivity of the assay was shown to be equivalent to a Real-Time PCR Assay for *Salmonella* in the food and environmental samples tested.

Significance: The results of these studies demonstrated that the novel system method is a fast, simple and accurate method for identifying *Salmonella* Enteritidis and Typhimurium in food and environmental samples. Furthermore, the assay shares identical PCR cycling parameters with other BAX® System real-time assays, allowing detection of multiple pathogens in a single PCR run in the BAX® System Q7 instrument.

P2-67 Comparative Validation Study to Demonstrate the Detection of *Listeria* spp. and *Listeria monocytogenes* in Fish and Seafood Products with Assurance GDS® for *Listeria* spp. and *Listeria monocytogenes*

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Introduction: Fish and seafood products have been identified as a source of past *Listeria monocytogenes* outbreaks. This study proposed a novel detection method, Assurance GDS, utilizing Immunomagnetic Separation (IMS) and Polymerase Chain Reaction (PCR) to detect *Listeria* spp. and *Listeria monocytogenes* in fish and seafood products in 28 hours or less.

Purpose: To demonstrate the equivalence of Assurance GDS for both *Listeria* spp. and *Listeria monocytogenes* Tq to the reference culture method for the detection of *L. monocytogenes* in fish and seafood products.

Methods: Four food matrices from the fish and seafood category representing smoked fish (smoked salmon), frozen fish (fish sticks), raw fish (cod fillets) and heat processed fish (canned tuna) were included in the study. For heat processed fish, 40 samples were inoculated with low levels of *L. monocytogenes*, 40 samples were inoculated with high levels of *L. monocytogenes* and 10 uninoculated samples were included as controls. For frozen and raw fish, 20 samples were inoculated with low levels of *L. monocytogenes*, 20 samples were inoculated with high levels of *L. monocytogenes* and 5 uninoculated samples were included as controls. 25-g test portions of heat processed and raw fish were enriched in 225 ml Buffered Listeria Enrichment Broth with Lithium Chloride and PALCAM supplement for 18 hours at 35-37°C. A 1 ml aliquot of the enrichment was transferred to 30°C an additional 6 hours. For frozen fish, 25-g test portions were enriched in 225 ml of Demi Fraser Broth for 22 hours at 30°C. All samples were analyzed using a novel method for detecting both *Listeria* spp. and *Listeria monocytogenes* according to the directions for use and reference culture methods. For all samples, the reference method used was Health Canada MFHPB-30.

Results: A total of 135 samples were analyzed for *Listeria* spp. a total of 180 samples were analyzed for *Listeria monocytogenes*. A probability of detection (POD) analysis showed that for all matrices, the performance of Assurance GDS for both *Listeria* spp. and *Listeria monocytogenes* Tq weres equivalent to the reference culture method.

Significance: This new detection method for fish and seafood provides the industry with a faster option for testing for both *Listeria* spp. and *Listeria monocytogenes* than culture methods, while retaining the necessary accuracy.

P2-68 Comparative Validation Study to Demonstrate the Detection of *Salmonella* in Cocoa and Chocolate-containing Products with Assurance GDS® *Salmonella* Tq

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Introduction: Traditionally, cocoa and chocolate products are considered difficult food matrices due to cocoa's dark color and natural inhibitory compounds. This study proposed a novel detection method, Assurance GDS, utilizing Immunomagnetic Separation (IMS) to isolate *Salmonella* from cocoa-based food samples containing these inhibitory compounds.

Purpose: To demonstrate the equivalence of Assurance GDS for *Salmonella* Tq to the reference culture methods for the detection of *Salmonella* in selected cocoa and chocolate containing foods.

Methods: Three food matrices (cocoa powder, milk chocolate and chocolate cake) representing the chocolate and confectionaries category were included in the study. 20 cocoa powder samples were inoculated with low levels of *Salmonella*, 40 samples were inoculated with high levels of *Salmonella* and 10 uninoculated samples were included as controls. 20 milk chocolate and chocolate cake samples were inoculated with low levels of *Salmonella*, 20 samples were inoculated with high levels of *Salmonella* and 5 uninoculated samples were included as controls. 25 g samples of cocoa powder and milk chocolate were enriched with a 1:10 sample to media ratio in UHT milk + Brilliant Green media for 18 – 24h at 36°C. 25 g samples of chocolate cake were enriched with the same ratio, temperature and time but in Buffered Peptone Water (BPW). All samples were analyzed using Assurance GDS for *Salmonella* Tq according to the directions for use and reference culture methods. For all samples, the reference method used was ISO 6579:2002.

Results: After analysis the data showed that for all three cocoa containing food matrices, McNemar's chi-square showed the performance of Assurance GDS *Salmonella* was equivalent to the reference culture method.

Significance: This new detection method for cocoa-containing foods gives customers a faster option for testing *Salmonella* than culture methods, while retaining the necessary accuracy for this difficult food matrix.

P2-69 Withdrawn

P2-70 Mechanism of *Staphylococcus aureus* Invasion into Caco-2 cell

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Introduction: *Staphylococcus aureus* is known to cause foodborne illness by intoxication rather than infection, but some strains of *S. aureus* are able to invade Caco-2 cells, which probably cause infection.

Purpose: The objective of this study was to elucidate the invasion mechanism of *S. aureus* into Caco-2 cell.

Methods: Five *S. aureus* strains (*S. aureus* KACC11596, *S. aureus* KACC10768, *S. aureus* KACC10778, *S. aureus* KACC13236 and *S. aureus* NCCP10862) were examined for Caco-2 cell invasion, and one strain, which invaded Caco-2 cell, was selected. The strain was then analyzed with iTRAQ (isobaric tags for relative and absolute quantification) to identify proteins related to Caco-2 cell invasion. The genes, which express the indentified proteins, were searched, and a mutant strain was prepared by knocking out genes using pIMAY and *E. coli* DC10B strain. Eventually, invasion efficiency was compared between the wild type and the mutant type of *S. aureus*.

Results: Invasion efficiency of five strains were compared, and *S. aureus* KACC10768 had significantly higher ($P < 0.05$) invasion efficiency than other strains. The result of iTRAQ analysis showed that pyruvate formate lyase (Pfl) was upregulated in *S. aureus* KACC10768 compared to other strains. This

result suggested that Pfl was the most frequent protein related to the invasion of *S. aureus* KACC10768. Thus, *pflB* gene of *S. aureus* was mutated, and *S. aureus* KACC10768 Δ*pflB* mutant had decreased Caco-2 cell invasion efficiency by 35% compared to the wild type.

Significance: This result indicates that *S. aureus* having high expression of *pflB* gene may cause foodborne illness by infection.

P2-71 Genotypic Characterization of Methicillin-sensitive and -resistant *Staphylococcus aureus* (MSSA/MRSA)

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Introduction: Although methicillin-resistant *Staphylococcus aureus* (MRSA) is the major source of hospital and community acquired infections, studies over many years have found MRSA in a variety of food products including retail meats and raw milk. These isolates cause more life-threatening infections compared to methicillin-sensitive *S. aureus* (MSSA). It is possible that people who handle such foods are prone to acquire MRSA.

Purpose: The purpose of this study was to determine the diversity and distribution of various virulence factors in MRSA and MSSA isolates.

Methods: In this study, we have compared a wide range of extracellular enterotoxin genes and virulence factors of 2 MSSA and 17 MRSA isolates from Pakistan. Chromosomal DNA isolated from bacterial cultures grown in Mueller-Hinton broth at 35°C were subjected to the pulsed-field gel electrophoretic (PFGE), PCR analysis, and sequencing and comparison of *spa* gene PCR products with known spa-types.

Results: Seventeen MRSA and two MSSA isolates exhibited 10 different PFGE patterns. A group of six and five MRSA isolates represented two major PFGE groups, respectively, indicating their clonal selection. PCR analysis of the 11 adhesin genes (*fnbA, fnbB, clfA, clfB, can, sdrC, sdrD, sdrE, bbp, ebpS,* and *map-eap*) indicated the presence of 7 common genes (*bbp, cna, clfA, clfB, fnbA, fnbB,* and *map-eap,*) in both, MRSA and MSSA isolates. Of the 19 toxin genes tested (*edin*, *eta, etb, hla, hlb, hld, hlg, hlg2, pvl, sea, seb, sec, sed, see, seg, seh, sei, sej,* and *tst*), 10 genes (*hla, hlb, hld, hlg, pvl, sed, see, seg, seh, tst*) were common in both, the MRSA and MSSA isolates. Five other virulence genes (*arcA, cfb, chp, ica,* and *v8*) were tested and only two (*cfb, v8*) were common among all the isolates. The spa-typing revealed 8 different spa-types, six of the MRSA strains could not be typed. The findings indicated that majority of the virulence gene markers were commonly present in MSSA and MRSA isolates.

Significance: Distribution of the virulence gene patterns and the prevalence of these genes in MSSA and MRSA isolates aids in our understanding of why MRSA strains are more pathogenic than MSSA strains. The results of this investigation will also be helpful in controlling future *S. aureus* outbreak infections.

P2-72 Application of Surface Sampling for the Monitoring of Human Norovirus on a Cruise Ship

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Introduction: Contaminated surfaces are regarded as a key vehicle to spread human noroviruses in semi closed settings such as cruise ships and healthcare facilities. However, little is known about the level of norovirus contaminated on hard surfaces during norovirus illness

Purpose: Determine norovirus contamination on environmental surfaces on a cruise ship

Methods: During a 5-day voyage of cruise ship A, several suspected cases of norovirus illness were reported. Within 2 hours after disembarkation of the passengers, environmental swab samples were collected from hard surfaces of several cabins from ill passengers as well as from surfaces in public places. Samples were shipped overnight on dry-ice and stored frozen until processing. Virus was eluted from the swabs and extracted using a newly developed norovirus extraction protocol. Extracts were tested for GI/GII norovirus by multiplex real time RT-PCR assay with coliphage MS2 included as a process control.

Results: A total of 92 swab samples were collected of which 17 (18%) tested positive for GII norovirus. Samples of 3 of the 6 cabins of symptomatic passengers tested positive including 50% of the restrooms. The norovirus load in the samples ranged from 16 - 31,217 RNA copy numbers. Overall, the viral load in the samples from the cabins (range 80 to 31,217) was higher than in the samples collected from the public spaces (range 16 to 113 RNA copy numbers). Four (23.5%) of positive swab samples could be sequenced and had identical sequences which could be genotyped as GII.1.

Significance: The viral load of the positive swab samples correlated with the location of known symptomatic norovirus passengers on the ship. Focusing environmental sampling on restrooms as index locations may provide a sensitive monitoring tool to identify norovirus contamination in high-risk settings to help guiding strategies to control norovirus.

P2-73 Detection of Norovirus in Relayed Oysters Implicated in an Outbreak

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Introduction: A significant portion of infectious foodborne illnesses in the US has been attributed to human enteric viruses, with norovirus (NoV) being the most common etiological agent identified in shellfish-associated viral gastrointestinal illnesses.

Purpose: The objectives were to concentrate, extract, detect and characterize norovirus viruses in oysters implicated in an outbreak.

Methods: Five bags of oysters were received and analyzed for NoV genogroups I and II, male specific coliphage (MSC) and coliforms. An ultracentrifugation protocol, with the inclusion of murine norovirus (MNV) as an extraction control, was used for concentration of enteric viruses from the implicated shellfish samples. For extraction and detection, Qiagen's ® silica-based protocol and a real-time RT-qPCR assay was used, respectively. Conventional RT-PCR and big-dye terminal sequencing was used to generate amplicons for characterization of norovirus. MSC were enumerated by a double agar overlay technique while coliforms were enumerated using the APHA MPN protocol.

Results: Levels of MSC, fecal coliforms, and *E. coli* in the oysters were <11 PFU/100g, 9.3-14 MPN/ 100g and 2.0- 4.5 MPN/ 100g, respectively. Only NoV genogroup I was detected with an average level of 2972 RT-PCR units/100g of digestive diverticula. Extraction efficiency of MNV from oysters was 85%. Sequence analysis and genotyping of the 329 bp fragment for norovirus revealed sequence 100 % homology to NoV and was characterized as NoV genogroup I.4. Norovirus genogroup I.4 was also identified in the clinical samples associated with this outbreak.

Significance: In the absence of cell culture techniques for the propagation of norovirus, the ability to detect and characterize norovirus in foodborne associated outbreaks was an integral part of this outbreak investigation. Detecting and characterizing GI.4 in the shellfish and clinical samples created the integral link between the consumer and the implicated shellfish.

P2-74 Inactivation of Tulane virus (TV) and Murine Norovirus 1 (MNV-1) by Electron Beam Irradiation

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Developing Scientist Competition

Introduction: Ionizing radiation, whether it is generated by electron beams (e-beams) or gamma rays, is an effective means of extending shelf life and enhancing microbial safety in foods. The mechanism of viral inactivation by gamma irradiation was recently demonstrated, but viral inactivation by e-beam remains to be investigated.

Purpose: One major objective was to evaluate the effect of electron beam irradiation on a new novel surrogate for human norovirus, Tulane virus (TV) in both liquid and food systems. Another key objective was to evaluate the mechanism of inactivation of two human norovirus surrogates (MNV-1, TV).

Methods: Tulane virus (TV) was inoculated onto samples of fresh cut strawberries or romaine lettuce, or diluted in simple solution (PBS) or complex solution (cell culture media). The samples were treated with electron beam irradiation (0-30 kGy), stomached in PBS, and the amount of surviving virus in fresh produce was quantified by plaque assay. A likely mechanism of inactivation was based on evidence from electron microscopy, RT-PCR, SDS-PAGE, and Western blotting.

Results: Electron beam irradiation was able to give a complete reduction of TV in both types of liquid, and both foods, but these results were at doses higher than currently permissible by the FDA (> 4 kGy).

Significance: This is the first published report on the inactivation of norovirus by electron beam irradiation. This is also among the first studies with a new novel surrogate, Tulane virus, believed to be the most behaviorally and genetically similar surrogate to human norovirus. Such results are of great value in studies to control human foodborne illness.

P2-75 Analysis of the Environmental Persistence of Tulane Virus, a Novel Cultivable Surrogate for Human Norovirus

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Introduction: Human noroviruses (HuNoV) are the most common cause of acute viral gastroenteritis and the leading cause of foodborne disease. Their environmental persistence is an important feature that influences their transmissibility by foodborne routes. Because HuNoV cannot be cultured *in vitro*, cultivatable surrogate viruses are often used to predict their behavior, although such predictions are not always accurate. Tulane virus (TV), which has features more similar to HuNoV than do other surrogates, is a promising alternative.

Purpose: To evaluate the environmental persistence of TV using infectivity assay and reverse transcription-qPCR (RT-qPCR), as appropriate.

Methods: TV was inoculated onto stainless steel coupons that were held at room temperature for up to 42 days. Virus inoculum was periodically recovered by elution, followed by RNA extraction (with and without prior RNase treatment to serve as proxy for infectivity) and RT-qPCR (for quantification of genome copy number). Parallel infectivity assays were done using the 50% Tissue Culture Infectious Dose ($TCID_{50}$) method.

Results: By RT-qPCR, there was approximately a 2-log reduction in the concentration of TV over 42 days on stainless steel. There was no statistically significant difference in virus titer when comparing RNase-treated to non-treated samples. A similar 2-log reduction in virus titer over 42 days was observed for TV using the $TCID_{50}$assay. No statistically significant differences between RT-qPCR and infectivity assay results were observed.

Significance: Similar to previous studies with HuNoV on surfaces, TV showed a high degree of environmental persistence. The lack of statistically significant differences when comparing persistence by RT-qPCR and infectivity assay is unique, as persistence is almost always greater when measured by RT-qPCR. TV is a promising surrogate for HuNoV in environmental persistence studies.

P2-76 Pulsed Light Inactivation of Murine Norovirus on Various Food Contact Surfaces

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Developing Scientist Competition

Introduction: Pulsed light is a promising non-thermal, surface decontamination technology, which has the potential to be applied in a food service setting. The effect of pulsed light on bacterial inactivation has been described in the scientific literature, but little information exists on the effect of the treatment on enteric viruses.

Purpose: The purpose of this work was to determine the effect of material type, treatment time, and distance from sample to the lamp on enteric virus inactivation by pulsed light.

Methods: Various materials common in food service settings, including 304 stainless steel, nitrile disposable gloves, and Romaine lettuce squares were each inoculated with 0.1 ml MNV-1 to achieve 7-log PFU/surface. Materials were treated with pulsed light in a Xenon Steripulse XL-3000™ pulsed light treatment system for up to 10 s, at a distance of either 83 or 133 mm from the central axis of the lamp.

Results: At a sample distance of 83 mm, MNV-1 was inactivated by 1.8-, 2.6-, and 3.9-log PFU/surface, after 10 s treatment on inoculated nitrile gloves, stainless steel, and Romaine lettuce, respectively. Distance from the lamp affected reduction, with significantly more reduction ($P > 0.05$) achieved after 10 s treatment at the shorter distance on nitrile gloves and stainless steel, but not for lettuce.

Significance: A relatively short treatment using pulsed light is sufficient to inactivate MNV-1 on the surface of materials commonly used in food preparation. The results suggest that the technology has the potential to reduce surface viral contamination in a food preparation setting.

P2-77 Human Norovirus GII.4 Strain Does Not Replicate in CV-1 Cells or HAV-persistently Infected A549 Cells

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Introduction: The successful cultivation of food-borne viruses is a high-priority research outcome with respect to food safety. An effective cell culture model for noroviruses (NoV), the most common agent of non-bacterial gastroenteritis in humans, has been unattainable despite numerous

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attempts in many cell lines. The lack of suitable in vitro culture methods for NoV has lent impetus to efforts in this field, as it would vastly improve food-related surveillance, epidemiology, and trace-back studies.

Purpose: The objective of this investigation was three-fold: (1) to determine if NoV will replicate in the African green monkey kidney cell line, CV-1, (2) to determine whether Hepatitis A virus (HAV) can function as a helper virus to support NoV replication in A549 cells (human lung carcinoma) persistently infected with HM175/clone 1 strain of HAV, and (3) to determine if incubation of these post-infected (NoV) cells at reduced cell density/ reduced temperature can promote NoV cpe/replication, as we have shown previously for persistently HAV-infected A549 and FRhK-4 cells.

Methods: Human stool, positive for NoV, was prepared as 10% clarified suspension in PBS, filtered, and quantified by qRT-PCR, and cell cultures were infected at an "moi" of 30 viral genome copies/cell. Post-adsorption supernatants were collected immediately after initial infection (2h at 37°C). Cell pellets and culture supernatants from cells incubated at 37 and 33°C were collected every week up to 7-8 weeks post-infection, pellets extracted for viral RNA, and tested for NoV, HAV, and non-NoV/non-HAV enteric virus replication using RT-qPCR.

Results: Input virus and post-adsorption supernatants tested positive for NoV, while all other samples were negative.

Significance: Reduced temperature and/or persistent (HAV) infection did not function to support NoV virus replication suggesting: neither cellular nor viral factor(s) in these cell lines, that contribute to the augmentation of HAV replication/cpe under similar incubation conditions, function to support NoV replication.

P2-78 Comparison of Thermal and Pressure-assisted Thermal D-values of *Clostridium botulinum* and *Clostridium sporogenes* PA3679 Spores

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Introduction: Impact of High Pressure Processing (HPP) on the survival of proteolytic spores of *Clostridium botulinum* is important in low-acid foods.

Purpose: Study and compare thermal and pressure-assisted thermal resistances of the most resistant proteolytic *C. botulinum* strains and *C. sporogenes* PA 3679 spores suspended in ACES buffer (pH 7.0).

Methods: Spores of Giorgio-A and 69-A strains of *C. botulinum* and *C. sporogenes* PA 3679 were prepared using biphasic media and diluted in ACES buffer (0.05M, pH 7) to 10^5-10^6CFU/ml. Diluted samples were placed into a modified sterile transfer pipette, heat-sealed and subjected to combinations of temperature (93-108°C) and high pressure (600-750 MPa) in a laboratory scale high pressure system. Another set of diluted spores were placed in NMR tubes, both ends heat-sealed, and subjected to various temperatures (93-108°C) and times in a Fluke 7321 High Precision Bath with Duratherm S as heat transfer fluid. Survivors were determined by a 5-tube MPN method using TPGY broth after incubation for 3 months.

Results: Thermal and pressure-assisted D-values (min) of Giorgio-A, 69-A, and PA3679 decreased as process temperature increased. Highest log reduction (> 4.5) of spores occurred at 108°C and the highest temperature and pressure combination (108°C and 750 MPa). Thermal D-values were higher for Giorgio-A, 69-A, and PA 3679 at any temperature compared those processed at the same temperature combined with pressure. For example, thermal D-values at 108°C for Giorgio-A, 69-A, and PA3679 were 3.5, 2.9, and 12.5 minutes, respectively. These times are higher than those obtained at the same temperature combined with 600 MPa (e.g. 2.4 for Giorgio-A, 2.1 for 69-A, and 5.7 for PA3679). Thermal and pressure-assisted D-values for PA3679 were higher than those for *C. botulinum* under the same conditions.

Significance: *C. sporogenes* PA3679 appears more resistant to thermal and pressure-assisted HPP than *C. botulinum* strains based on the thermal and pressure-assisted D-values.

Poster

P2-79 Characteristics of *Clostridium perfringens* from U.S. Foodborne Outbreaks

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Introduction: It is estimated that one in six Americans or 50 million people attract a foodborne illness each year in the United States. Of these, *Clostridium perfringens* is estimated to be responsible for 1 million. This pathogen induces foodborne illness by expression of the *C. perfringens* enterotoxin gene, *cpe*.

Purpose: The purpose of this study was to evaluate the presence/absence of enterotoxin genes and genetic characteristics of *C. perfringens* recovered from foodborne outbreaks in the US.

Methods: We evaluated 90 isolates from 18 outbreaks representing 13 states and one cruise ship. Isolates were genotyped for the *cpe* gene by PCR and draft genome sequences from representative isolates were generated using the Ion Torrent PGM. Draft genomes were compared using reference-free SNP analysis.

Results: Of 90 isolates, 56 (62%) harbored the *cpe* gene indicating potential to cause diarrheal illness. Notably, 34 (38%) of the isolates did not carry the gene and were incorrectly identified as the causative agent of the outbreak. The genome sequences of two *cpe*-positive isolates were more closely related to each other than the genomes of two *cpe*-negative isolates. Moreover, the sequences of *cpe*-positive and *cpe*-negative strains isolated from the same outbreak did not cluster together.

Significance: Toxin-negative *C. perfringens* is commonly attributed to the etiology of foodborne illness; however, genome sequencing reveals that such isolates are unlikely to be related to the etiological agent. All isolates should be screened for the enterotoxin gene and ideally, for presence of the toxin in stools. Furthermore, genome sequences of *C. perfringens* isolated from individuals with foodborne illness can be used to rapidly distinguish pathogenic isolates from commensal isolates which is important for comparison with isolates from foods or other individuals involved in an outbreak.

P2-80 Fate of *Clostridium difficile* in Sewage Waste Water Treatment and Biosolids Amended Soil

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Introduction: There have been an increasing number of Community Associated *Clostridium difficile* infections across developed and developing nations although the primary source of the pathogen has yet to be elucidated. Foodborne and zoonotic transfer has been suggested although considered minor sources by which the population is exposed to *C. difficile*. The role of environmental exposure (e.g. recreation waters, soil) in disseminating *C. difficile* has yet to be considered and hence requires to be evaluated.

Purpose: The following study aimed to determine the incidence of *C. difficile* in sewage and the fate of the pathogen during sludge digestion and subsequent disposal of the effluent/biosolids into the environment.

Methods: A total of 59 biosolids samples from waste water treatment plants in Ontario were collected and *C. difficile* was isolated. Toxigenic profile (toxin A, toxin B, and binary toxin) and genotypes (PCR ribotyping, toxinotyping and pulsed field gel electrophoresis) of each isolates were

determined. The survival ability of ribotype 078 (5 log CFU/ml) and ribotype 027 (6 log CFU/ml) in biosolids-amended soils (sandy and loam) in farms was determined, sampling was carried out once a month.

Results: *C. difficile* was found in 72.9% (43/59) biosolids samples and 35 (81.4%) of the isolates were toxigenic while all of the toxigenic isolates possessed *tcdA* and *tcdB* and 17 (48.6%) also possessed *cdtA*. Fifteen of the 17 (88.2%) A+B+CDT+ isolates were classified as PCR ribotype 078, toxinotype V, 13 of them were NAP7 and 2 were NAP8. From May to Nov 2013, ribotype 027 in sandy and loam soils stayed stable around 6 log CFU/ml. Ribotype 078 in sandy soil remained steadily around 5 log CFU/ml with a sharp decrease to 4 log CFU/ml in August and back to 5 log CFU/ml in September again. In loam soils, ribotype 078 declined sharply from 5 to 1 log CFU/ml from May to August and rose back to 4 log CFU/ml in September followed with slightly decrease.

Significance: Toxigenic *C. difficile* is highly prevalent within raw sewage and survives the waste water treatment process. The effluent and biosolids resulting from sewage treatment represents a significant environmental burden of *C difficile*.

P2-81 Canada's National Food Microbiological Monitoring Program

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Introduction: The Canadian Food Inspection Agency (CFIA) is Canada's federal food safety, animal health and plant protection enforcement agency. One of the roles of the Agency is to monitor and regulate Canada's food supply to ensure safety and the enforcement of standards. This is achieved through a series of activities that range from the inspection of federally-registered establishments to border inspections, laboratory testing and surveys, the performance of food safety investigations, risk assessments, and regulatory action on unsatisfactory results.

Purpose: The National Microbiological Monitoring Program (NMMP) is one of many tools utilized by the CFIA to verify that both domestically produced and imported food products meet Canadian standards. It is designed to sample and test for the presence of pathogens in foods deemed to pose the greatest risk to consumers, and covers a broad range of domestic and imported foods of both plant and animal origin.

Methods: The NMMP consists of a variety of microbiological sampling activities such as (i) monitoring by random sampling of the food supply to verify compliance, and (ii) risk-based sampling through enhanced sampling of specific food/hazard combinations that are of greater concern to human health.

Results: Results from this testing enable the CFIA to make decisions concerning the acceptability of food based on its microbial quality.

Significance: A summary of the sampling and testing activities performed under the NMMP over the 2011/12 to 2013/14 time period will be presented.

P2-82 All over the Map: A Review of Foodborne Illness Investigation in Fifty States

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Introduction: State-level public health agencies provide the raw data necessary for attribution research—but not all agencies are equal. For this study, foodborne outbreak data from 50 states were analyzed to determine the success rate of investigations in each state. States are ranked, and results benchmarked against states with historically robust investigation results.

Purpose: This study aims to evaluate the relative performance of state-level public health departments to investigate foodborne illness outbreaks, and provide essential information for downstream attribution research. Identifying successful state programs can help identify best practices to advance agencies' public health mission.

Methods: Foodborne outbreak data submitted by states to the Centers for Disease Control and Prevention (CDC) was acquired from CDC for the years 2002-2011. Outbreaks were sorted into two categories: (1) attributable (where both the food and pathogen were identified by epidemiologists), and (2) non-attributable (where data were missing). Those data were sorted by state, and a comparative metric was created.

Results: Our results identify and explore variation in state-level reporting that impact the quality of outbreak investigations and the evidence needed for food attribution. States with successful and deficient systems are identified, based on 10 years of reporting data. CSPI identifies best practices that might contribute to successful programs.

Significance: This research demonstrates the importance of state-based public health reporting practices. By highlighting best performing states, we hope to identify policies that enable best practice operations for health departments.

P2-83 Estimating the Burden of Foodborne Illness in Japan Using Clinical Laboratory Data for Whole of Japan, 2006-2011

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Introduction: In Japan, the numbers of food poisoning and cases are reported mandatory; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive surveillance nature. We have been estimating the real burden of diarrheal diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan.

Purpose: The purpose of this study was to estimate the burden of foodborne illnesses associated with three pathogens for whole of Japan, by making use of the laboratory confirmed numbers of infections.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories that test stool samples submitted from all over Japan or from Miyagi Prefecture, from January 2006 to December 2011. The stool submission rate and the physician consultation rate were estimated from telephone population surveys conducted for Miyagi prefecture and for whole of Japan. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in whole of Japan were 3.4-5.3 million, 0.59-1.1 million, and 44-170 thousand during 2006-2011, respectively. Those estimated for whole of Japan from data on Miyagi prefecture were 0.93-1.6 million, 78-264 thousand, and 14-83 thousand during 2006-2011, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006-2011, for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus*, were 2,100-3,100, 1,500-3,600 and 90-1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuous active surveillance system to complement the present

passive surveillance is strongly suggested, in order to identify and prioritize food safety issues more precisely and to monitor the effectiveness of risk management options.

P2-84 Frequency and Antimicrobial Resistance Patterns of *Salmonella* in Mixed Crop-Animal Farms and Its Products in Retail Stores and Farmers' Markets

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Introduction: *Salmonella* is estimated to be the second highest causative bacterial agent of foodborne illness. More outbreaks have been attributed to vegetables, specifically organic products. Mixed crop-animal/back yard farms, possessing higher biosecurity and biosafety risks, are one of the major producers of organic products, supplying farmers markets as well as organic stores. It is essential to understand possible ecological sources of contamination at the production level and the translation of contamination at the consumption level.

Purpose: To investigate the frequency and antimicrobial susceptibility of *Salmonella* in vegetable and environmental samples at the production level as well as in post-harvest level.

Methods: Samples (n = 350) were collected from organic farms, farmers markets, organic and conventional supermarkets located in Washington D.C. or Maryland. Portions of each sample were diluted 1:9 (wt/vol) with buffered peptone water. After enrichment in Luria-Bertani broth containing 5% sheep blood, samples were streaked on XLT-4 agar; presumptive colonies were confirmed biochemically and through PCR analysis. Antimicrobial susceptibility testing was carried out using the agar dilution method described by the Clinical Laboratory Standards Institute. The data was analyzed using the χ^2 test.

Results: On organic farms surveyed, there was a significant ($P = 0.01$) difference in the occurrence of *Salmonella* between environmental, animal feces, animal feed and vegetable samples, with prevalence of 35%, 70%, 22% and 20%, respectively. There was no significant difference in the rates of antimicrobial resistance of *Salmonella* recovered from those environments. Further there was no significant difference in *Salmonella* prevalence and antimicrobial resistance between vegetables from organic markets, conventional markets, farmers markets and farms.

Significance: While the findings show contamination at the farm level is significantly different, factors contributing to antimicrobial resistance of vegetables were not clearly identified. These data also suggest that there is no difference in microbiological quality of organic vegetables compared to conventionally raised vegetables.

P2-85 Dynamics of *Campylobacter* in Mixed Crop-livestock Farms and Cross-contamination of this Pathogen in Its Products

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Introduction: In mixed crop-livestock farms, the farmers compost the animal waste and use it to grow produces including lettuce, spinach, tomatoes, green pepper and cantaloupe. These crops can be vehicles for a variety of enteric bacterial pathogens including *Campylobacter*. The source of contamination of fresh produce with enteric pathogens can frequently be traced back to environmental reservoirs associated with farm animals. Environmental factors and farming practices can affect transmission of enteric foodborne pathogens from animal reservoirs to leafy greens via soil, water and vectors.

Purpose: The purpose of this study was to determine the ecological surveillance of *Campylobacter* in mixed farm environments and its products at pre- and post-harvest levels.

Methods: Total 584 samples from five organic farms and three conventional chicken farms from Maryland and Washington DC area were collected and analyzed. For post-harvest analysis, ready to sale products were collected from five farmers markets and four chain shops in the same area. *Campylobacter* was identified and characterized with biochemical tests and PCR.

Results: *Campylobacter* prevalence in fecal samples were 36% (18/50) and 9% (9/100); in environmental samples 16.13% (10/62) and 3.70% (3/81); in feed & water samples 11.76% (8/68) and 6.25% (5/80) in organic and conventional farms, respectively. Produce samples grown in organic mixed crop-livestock farms and only organic produce farms had prevalence rate of 15.56% (7/45) and 18% (9/50), respectively, whereas no positive samples were found from conventionally grown produce (0/20). *Campylobacter* prevalence in produce samples collected from farmers market was 4.11% (9/219) but no positive (0/80) sample was found from conventional retail shop.

Significance: These data suggest that prevalence of *Campylobacter* in produces is higher in organic mixed crop-livestock farms and its products in both pre- and post-harvest levels. Natural organic antimicrobial agents and better practices are essential to control cross-contamination of pathogens.

P2-86 Prevalence of Enterohemorrhagic *Escherichia coli* on Hides of Beef Feedlot Cattle

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Developing Scientist Competition

Introduction: Intimin-positive serogroup O26, O45, O103, O111, O121, O145, and O157 Shiga toxin-producing *E. coli* have been declared adulterants in non-intact, raw beef by the USDA-FSIS. These organisms, also classified as enterohemorrhagic *E. coli* (EHEC), are spread to carcass surfaces from hides during hide removal at harvest.

Purpose: The objective of this study was to determine the prevalence of the 7 adulterant types of EHEC (EHEC-7) on the hides of commercial feedlot beef cattle at harvest.

Methods: Twenty-four pens of crossbred finishing cattle from a large commercial feedlot in the Central U.S. were sampled at harvest using a repeated cross-sectional study design from June to August 2013 (24 cattle/pen, 2 pen/week, 12 weeks; n=576). Hide swab samples were enriched in EC broth, subjected to immunomagnetic separation (IMS), and plated on modified Possé agar. Isolates were tested by multiplex PCR for genes for O-group, Shiga toxin 1 and 2, intimin and enterohemolysin, and DNA from an aliquot of enriched broth was tested by the NeoSEEK™ STEC Detection and Identification test (Neogen®Corp.).

Results: By NeoSEEK™, the prevalence was EHEC-7, 98.1%; EHEC O26, 0.5%; EHEC O45, 40.8%; EHEC O103, 97.2%; EHEC O111, 4.5%; EHEC O121, 2.4%; EHEC O145, 49.1%; and EHEC O157, 14.4%. By culture, the prevalence was EHEC-7, 1.5%; EHEC O26, 0.6%; EHEC O145, 0.2%; EHEC O157, 0.6%; and EHEC O45, EHEC O103, EHEC O111, and EHEC O121, 0.0%.

Significance: Based on the NeoSEEK™ test, the prevalence of EHEC-7 on hides of feedlot beef cattle was extremely high, nearly 100%, with EHEC O103, O145, O45, and O157 the most prevalent. By comparison, the prevalence based on culture was very low, with EHEC O26 and O157 in highest prevalence, followed by EHEC O145. The data collected in this study will be used to populate a quantitative microbial risk assessment model for these pathogens along the beef chain.

P2-87 Comparative Genomic Fingerprinting of *Campylobacter jejuni* Strains Isolated from Poultry and Clinical Patients in Atlantic Canada

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Introduction: *Campylobacter* spp. are currently the leading cause of foodborne gastroenteritis in Canada, the US and many regions of Europe. Raw, retail poultry is a significant source of *Campylobacter*. However in Canada, there is limited information about whether strains carried on poultry actually cause disease in humans.

Purpose: To strain type *Campylobacter jejuni* and *C. coli* isolates recovered from raw, retail poultry, as well as clinical isolates collected from hospitals in Atlantic Canada, to determine whether certain *Campylobacter* types are found concurrently in both retail poultry and clinical patients.

Methods: Thirty packages of raw, retail poultry sold in Halifax, Nova Scotia, were tested for *Campylobacter* weekly between July and October (n = 480) using a modified Bolton broth method. Clinical isolates were obtained retrospectively and prospectively from hospital laboratories in the Atlantic region. *C. jejuni* and *C. coli* isolates were typed using comparative genomics fingerprinting (CGF). Select isolates were further compared using whole genome sequencing.

Results: We isolated *Campylobacter* spp. from 65% of retail packages and 285 of the total 312 isolates (91%) were identified as *C. jejuni*. The type of meat cut, presence of skin/bone or time of sampling did not significantly affect the frequency of recovery. Currently, 123 strain types have been identified. Of these, 49 types formed clusters (i.e., identified in more than one isolate). Of these clusters, 32 (26% of the total types) present in clinical patients and retail poultry, 3 (2.4% of the total types) present in only clinical patients and 14 (11.4% of the total types) present in only retail poultry. The remaining 74 (60% of the total) did not form clusters.

Significance: These results demonstrate that raw, retail poultry can be a source of *Campylobacter* strains carried in clinical patients. The results also suggest that not all poultry-associated *Campylobacter* strains are implicated in human disease.

P2-88 International Divergence of Bovine and Human Shiga Toxin-producing *Escherichia coli* O157:H7 Genotypes

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Developing Scientist Competition

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a zoonotic pathogen of significant public health concern in many countries. Ruminants are asymptomatic carriers of STEC, shedding the pathogen via faeces, and are recognised as an important source of infection in humans.

Purpose: To compare the genotype distribution of bovine and human *E. coli* O157:H7 isolates from New Zealand (NZ), Australia (AU), and the United States (US) using Shiga toxin-encoding bacteriophage insertion (SBI) genotyping data, and assess evidence of *E. coli* O157:H7 transmission from cattle to humans for each country.

Methods: SBI typing is a multiplex PCR method for screening specific Shiga toxin (*stx*)-associated bacteriophage insertion sites and *stx* genes (*stx*1, and genetic subtypes *stx*2a and *stx*2c of *stx*2). The characters A, W, Y, S and 1, 2a, 2c represent bacteriophage insertion sites *argW*, *wrbA*, *yehV*, *sbcB*, and *stx* genes *stx*1, *stx*2a, *stx*2c, respectively. SBI types of 40, 205, and 143 bovine, and 363, 79, and 179 human *E. coli* O157:H7 isolates from NZ, AU, and the US, respectively, were evaluated. Proportional similarity indices (PSI) were computed based on the frequency distributions of SBI types to illustrate the epidemiological linkage between bovine and human isolates for each country.

Results: A distinct prevalence distribution of SBI types was observed between the countries. AY2a was predominant among NZ bovine and human isolates (55.0% and 57.9%, respectively), ASY12c was predominant among AU bovine and human isolates (51.7% and 43.0%, respectively), and WY12a was predominant among US bovine and human isolates (43.4% and 63.7%, respectively). NZ bovine and human genotypes shared the highest similarity (PSI value of 0.92) followed by AU (0.69) and the US (0.61).

Significance: Highly evident divergence of genotypes was demonstrated between NZ, AU, and the US but the reason for this is unknown. Possibilities include genetic drift and/or selection driven by different environmental factors, such as climate, types of feed, husbandry systems, or animal genetics. There was strong evidence for a close association between cattle and human populations of *E. coli* O157:H7 isolates in NZ, consistent with transmission of STEC from cattle to humans (or transmission to both hosts from another environmental reservoir).

P2-89 Validation of a New Enumeration Method for *Campylobacter* Based on a Chromogenic Media in Selected Food Matrices and Environmental Samples

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Introduction: Over the past several years, *Campylobacter* had been the most frequently reported causes of bacterial foodborne illness in the Europe and United States. *Campylobacter* are frequently isolated from foods of animal origin and the food manufacturing environment. The use of selective chromogenic agar increases the ease of use, with results based on color change enzymatic reactions.

Purpose: RAPID'*Campylobacter* is a media for the enumeration of thermophilic *Campylobacter* in food and environmental samples based on a chromogenic reaction. The use of a selected nutritive mixture associated with reducing agent allows the growth of *Campylobacter* spp. in an optimal time. Other bacterial species, as well as yeast and molds, are inhibited by the selective agents. *Campylobacter* spp. produces brick-red colonies on the media. A validation based on NF VALIDATION rules and ISO 16140 standard has been conducted by an independent expert laboratory (ADRIADéveloppement).

Methods: In the method comparison study, RAPID'*Campylobacter* was compared to the ISO/TS 10272-2:2006 method. Two food categories and one environmental matrix were tested. Three matrix/strain pairs were tested during the linearity study, and 120 samples were tested in the relative accuracy study. Inter-laboratory study was performed by 15 laboratories.

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Results: Method comparison results demonstrated there was no significant difference in the linearity, accuracy, specificity and sensitivity of the RAPID'*Campylobacter* method when compared to the ISO 10272-2 standard. Inter-laboratory results demonstrated equivalent precision of the two methods, in repeatability and reproducibility conditions with low bias.

Significance: The chromogenic substrate and selective component allow a high level of contrast and ensures an optimal reading of *Campylobacter*, which appears brick-red on a clear medium. The method presented greatly shortens the time to a *Campylobacter* enumeration by eliminating the need for long and difficult confirmation step, and reducing the laborious step required in traditional methods for *Campylobacter* enumeration.

P2-90 Validation of a New Enumeration Method for *Enterobacteriaceae* Based on a Combination of Color Indicators in Selected Matrices of Food

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Introduction: The members of the *Enterobacteriaceae* family constitute the most widely used indicators of hygiene. *Enterobacteriaceae* may contaminate food and equipment and monitoring is recommended in both the food manufacturing environment and the end product. The use of new solution, based on agar media using a combination of color indicators, increases the ease of use and reduces the time to result and allows the automation of the reading step.

Purpose: RAPID'*Enterobacteriaceae* is a media for the enumeration of *Enterobacteriaceae* in food based on the glucose fermentation. The combination of color indicators allows a high level of contrast of *Enterobacteriaceae* colonies which appear as red on a clear grey medium. A validation based on NF VALIDATION rules and ISO 16140 standard has been conducted using protocols without confirmation and using an automated colony counter providing a direct result in CFU/g.

Methods: In the method comparison study, RAPID'Enterobacteriaceae was compared to the ISO 21528-2 method. Four food categories were tested with two protocols (pour plate method and surface inoculation) and two possibilities of reading (using an automated colony counter or eye reading). Inter-laboratory study was performed by 17 laboratories.

Results: Method comparison results demonstrated there was no significant difference in the linearity, accuracy, specificity and sensitivity of the RAPID'*Enterobacteriaceae* method when compared to the ISO standard. Inter-laboratory results demonstrated equivalent precision of the two methods, in repeatability and reproducibility conditions and low bias between -0.023 log CFU/g and 0.038 CFU/g.

Significance: The method presented greatly shortens the time to *Enterobacteriaceae* enumeration by eliminating the need for a confirmation step. The combination of color indicators allows a high level of contrast and ensures an optimal reading. This feature enables the use of an automated colony counter providing a direct result in CFU / g and full traceability for the laboratory.

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P2-91 A New and Convenient Method (TEMPO® "CAM") for Enumeration of *Campylobacter* spp. from Poultry-associated Matrices

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Introduction: *Campylobacter jejuni* and *C. coli* are the leading causes of human foodborne bacterial gastroenteritis worldwide, with poultry meat almost exclusively implicated as the vehicle of transmission. Conventional procedures for the enumeration of *Campylobacter* spp., such as direct plating according to ISO 10272-2:2006, are time-consuming and resource-intensive. The TEMPO® system, which has been developed for other bacterial populations, is an easy-to-use automated enumeration technique based on most probable number (MPN) principles.

Purpose: To develop a prototype assay and compare to standard methods for the enumeration of *Campylobacter* in poultry matrices using the TEMPO® platform.

Methods: A proprietary medium was formulated, consisting of CampyFood Broth®, two additional selective antibiotics and a fluorescent indicator. Ninety-six samples were used for evaluation of the assay against ISO/TS 10272-2:2006 (mCCDA), including broiler chicken feces, ceca, neck skin and carcasses collected at different points along the processing chain. All cards and plates were incubated at 41.5°C for 48h under microaerobic conditions. Counts were converted to log cells/ml and analyzed statistically.

Results: A linear association ($R^2 = 0.96$; *t*-test $P \geq 0.05$) was observed between TEMPO® "CAM" and ISO/TS 10272-2:2006. Quantitative analysis showed 97% of enumerations were less than ± 1 log cells/ml different between the two methods. A sensitivity and specificity of 88% each, and good agreement (KAPPA = 0.62) were also determined.

Significance: In light of recent performance standards and objectives, which have set quantitative limits for campylobacters on processed dressed poultry carcasses, this study presents preliminary data for a sensitive, automated method for the enumeration of *Campylobacter*. Optimization and commercialization of TEMPO® "CAM" is in progress.

P2-92 Rapid and Simultaneous Detection of *Campylobacter* and *Salmonella* in Poultry Samples Using Magnetic Nanobeads and Quantum Dots Based Fluorescent Immunosensor

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Introduction: *Campylobacter* and *Salmonella* are the most important bacterial pathogens associated with foodborne diseases caused by consuming undercooked poultry or handling raw poultry and poultry products. *C. jejuni* and Salmonella cause 2.4 million and over 1 million cases of foodborne illness, respectively, in the United States each year. The development of rapid, sensitive, and specific methods for detection of food-associated bacterial pathogens remains challenging.

Purpose: To develop a sensitive biosensing method for rapid detection of *C. jejuni* and *Salmonella* simultaneously in chicken and ground turkey wash solutions using magnetic nanobeads to separate and concentrate the target bacteria and quantum dots (QDs) as fluorescent markers.

Methods: Both streptavidin conjugated QDs 530 and QDs 620 were separately coated with the specific biotin conjugated anti-*Salmonella* and anti-*C. jejuni* antibodies. The magnetic nanobeads also were separately coated with the specific biotin conjugated anti-*Salmonella* and anti-*C. jejuni* antibodies. The conjugated magnetic nanobeads then were mixed with a sample containing *Salmonella* and *C. jejuni*. After immunomagnetic separation, the magnetic nanobeads-*Salmonella* and nanobeads-*Campylobacter* conjugates were mixed with the conjugated QDs 530 and QDs 620 in the same test tube. Unattached conjugated QDs were removed using immunomagnetic separation. Fluorescence spectrometer at 530 and 620 nm was used to measure the complexes of magnetic beads–*S.* Enteritidis-QDs and beads-*C. jejuni*-QDs with a total detection time of less than 2 hrs.

Results: *C. jejuni* (QDs 620) and *S.* Enteritidis (QDs 530), in pure culture, chicken carcass and ground turkey wash solutions were simultaneously separated and detected. The fluorescence intensities increased significantly with the increasing cell number of both bacteria. The multiple detection limit was 20-50 CFU/ml and the detection time was less than 2 hrs.

Significance: This study would provide the poultry industry a more efficient rapid method for detection of major foodborne pathogens on products to ensure food safety.

P2-93 Optimizing a Culture Method for Detecting Non-O157 Shiga Toxin-producing *Escherichia coli* from Dairy Compost

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Developing Scientist Competition

Introduction: Foodborne illnesses, linked to non-O157 Shiga toxin-producing *Escherichia coli* (STEC), such as serotypes O26, O45, O111, O103, O145 and O121, have been increasing. Animal manure and compost appear to be the potential reservoirs for non-O157 STEC. To control STEC in biological soil amendment, sensitive and easy detection methods are needed.

Purpose: The objectives of this study were to optimize culturing methods for detecting STEC in dairy compost and also to compare growth rates of different STEC serotypes during enrichment of dairy compost.

Methods: The finished dairy compost with 30% moisture content was inoculated with a cocktail of six STEC serotypes at a final concentration of ca. 10^2 CFU/g. Afterwards, bacterial cells in the inoculated compost were enriched by four methods: pre-enrichment by universal pre-enrichment broth (UPB) followed by selective enrichment using STEC enrichment broth or modified Tryptic Soy Broth with novobiocin (mTSB+n), direct selective enrichment by STEC-EB or mTSB+n, and the enrichment efficiencies were compared by plating enriched cultures onto Cefixime-Tellurite Sorbitol MacConkey Agar with noviobiocin (CTN-SMAC) and modified Rainbow Agar (mRBA). Immunomagnetic separation (IMS) was applied to separate STEC serotypes after enrichment.

Results: There was no significant difference ($P > 0.05$) between CTN-SMAC and mRBA for STEC enumeration. Applying selective enrichment methods alone recovered ca. 0.5 log CFU/g more cells as compared to the two-step enrichment methods. Among six STEC serotypes, serotypes O45 and O145 grew faster and the cell populations reached up to 7.29 and 7.83 log CFU/g, respectively, after 16-hour enrichment, suggesting these two as the potential persistent serotypes surviving in dairy compost.

Significance: Our results demonstrated that low levels of STEC could be detected from dairy compost by culturing method through optimizing enrichment procedure followed by IMS, which can be useful to study the growth and survival of STEC during composting or in dairy manure-based soil amendments.

P2-94 A Matrix Extension and Method Modification of the 3M™ Molecular Detection Assay *Salmonella* for the Detection of *Salmonella* Species in a Variety of Foods and Environmental Surfaces

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Introduction: *Salmonella* has been implicated as a main causes of foodborne outbreaks and detection can be time consuming and expensive which results in a strong need for rapid and reliable methods. The 3M™ Molecular Detection Assay *Salmonella* (AOAC OMA 2013.09) is designed for the rapid and specific detection of *Salmonella* in enriched food, feed and environmental samples, utilizing a combination of isothermal amplification and bioluminescence. This method has been modified to expand its matrix claim and provide same day detection of *Salmonella* in certain food products.

Purpose: The purpose of this AOAC OMA matrix extension and method modification was to add 11 additional matrices to the method claim (raw ground chicken 25g & 375g, chicken carcass rinsates & sponges, pasteurized American cheese, dry dog food 375g, creamy peanut butter, raw head on shrimp, sprout irrigation water 375g, concrete, ceramic tile and stainless steel) and add a new enrichment protocol to the method: 10 hour enrichment at 41.5°C for raw ground beef (25g, 325g & 375g).

Methods: Each matrix was evaluated by the new method and the FDA/BAM or USDA/FSIS-MLG at the following inoculation levels: 20 replicates at 0.2-2 CFU/test portion, 5 replicates at 2-5 CFU/test portion, and 5 replicates at 0 CFU/test portion. New matrices were evaluated after 18 hours of primary enrichment. Raw ground chicken was analyzed at 14 hours for 325g and 10 hours for 25g test portions. Raw ground beef test portions were evaluated after 10 hours of enrichment at 41.5°C. **Results** for each matrix were analyzed using the POD statistical model.

Results: No statistically significant differences were observed between the new method and reference methods for each new food or for the method modification.

Significance: The new method demonstrated high sensitivity and specificity for the detection of *Salmonella* in the new matrices and with the new enrichment protocol.

P2-95 Evaluation of Two New Methods for the Detection of *Listeria* in 125-Gram Food Samples: Collaborative Study

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Introduction: The VIDAS® UP *Listeria* (LPT) and the VIDAS® *Listeria monocytogenes* Xpress (LMX) are automated rapid screening assays for the detection of *Listeria* species and *Listeria monocytogenes* in human food products. The LPT, an enzyme phage-ligand based assay and the LMX, an enzyme fluorescent immunoassay, utilize a harmonized enrichment protocol in a proprietary LPT broth for 125-gram test portions. These new methods were collaboratively studied to evaluate their performance in the detection of the target pathogen in larger sample sizes.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the two rapid methods to the AOAC OMA 993.12 for Soft Mexican Cheese (125g).

Methods: The two rapid methods were compared in a multi-laboratory collaborative study to the AOAC OMA 993.12 *Listeria monocytogenes in Milk and Dairy Products* reference method. A total of 14 laboratories participated, representing government and industry, throughout the United States. Soft Mexican cheese was analyzed using 125-gram sample size. Each replicate was artificially contaminated with *Listeria monocytogenes* ATCC 19115 at 3 levels, an un-inoculated control level (0 CFU/125 g), a low inoculum level (0.2-2 CFU/125 g) and a high inoculum level (2-5 CFU/125 g). All test portions

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were confirmed using Oxford agar, as prescribed by the reference method, as well as ALOA chromogenic agar for the identification and differentiation of *L. monocytogenes* and *Listeria* species.

Results: For this evaluation, 900 unpaired replicate test portions (125 g) were analyzed by either the candidate or reference methods. Each inoculation level was analyzed using the Probability of Detection (POD) statistical model. For both VIDAS LPT and LMX low level inoculated test portions, dLPOD values of 0.01, (-0.10, 0.13), with 95% confidence intervals, were obtained. The range of the confidence intervals for dLPOD values contain the point 0.0 indicating no statistically significant difference in the number of positive samples detected between the new methods and the AOAC reference method. Additionally, no differences were observed between the two selective agars used for confirmation.

Significance: The collaborative study of these two novel methods, along with the optional ALOA agar confirmation method, demonstrated their robustness and high level of reproducibility in the detection of *Listeria spp* in 125-gram food samples. The harmonized 125g enrichment protocol for LPT and LMX enables the detection of *Listeria spp* and *L. monocytogenes* from the same sample.

P2-96 Evaluation of the 3M™ Petrifilm™ *Salmonella* Express System for the Detection of *Salmonella* in Select Foods: Collaborative Study

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Introduction: The 3M™ Petrifilm™ *Salmonella* Express System (SALX) is a simple, ready-to-use chromogenic culture medium system for the rapid qualitative detection and biochemical confirmation of *Salmonella* spp. in food and environmental samples. This new method utilizes the proprietary 3M™ *Salmonella* Enrichment Base and 3M™ *Salmonella* Enrichment Supplement which allows for presumptive results in as little as 40 hours from low microbial background foods (<10^4 CFU/g) and 48 hours from high microbial background foods (≥10^4).

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the new method to the FDA/BAM for dry dog food (375g) and the USDA/FSIS-MLG for raw ground beef (25g).

Methods: This new method was compared in a multilaboratory collaborative study to the FDA/BAM Chapter 5 method and the USDA MLG 4.06 reference methods. A total of 17 laboratories located within the continental U.S participated. Each matrix was artificially contaminated with *Salmonella* at 3 inoculation levels: a low-level inoculum of 0.2-2 CFU/test portion, a high-level inoculum 2-5 CFU/test portion and an un-inoculated control level 0 CFU/test portion. All test portions were confirmed via traditional confirmation procedures and an alternative confirmation procedure directly from the 3M™ Petrifilm SALX Plate.

Results: In this study, over 500 samples were analyzed by the new method and the reference methods for both the raw ground beef and dry dog food. Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. No statistically significant difference was observed between the new and reference methods for both matrices. Additionally, no differences were observed between the alternative and traditional confirmation procedures for the new method.

Significance: The collaborative study demonstrated the reliability and sensitivity of the 3M™ Petrifilm *Salmonella* Express System for the rapid detection of *Salmonella* in food products using both 25-g and 375-g sample sizes.

P2-97 A Comparative Evaluation of Romer Lab's RapidChek *Listeria* F.A.S.T Environmental System for the Detection of *Listeria* on Stainless Steel Surfaces against a Variety of Rapid Methods

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Introduction: Outbreaks linked to *Listeria monocytogenes* cause grave concern to manufacturers and producers. Listeriosis, the disease caused by the bacterium, while rare, has a high mortality rate, especially among the younger, the elderly and the immuno-compromised. The food industry needs to have methods that can rapidly detect the organism, yet are sensitive enough to detect the pathogen in low quantities. The RapidChek *Listeria* F.A.S.T. Environmental System offers the benefits of a single, proprietary enrichment, coupled with innovative immuno-strips technology to allow processers and manufacturers the ability to detect the dangerous pathogen from food production surfaces in only 24 hours. The new method couples rapid and accurate results, without the need to purchase expensive capital equipment.

Purpose: The purpose of this evaluation was to conduct a method comparison on the new method, along with two commercially-available enzyme immunoassays and two PCR assays, for the detection of *Listeria* spp. on stainless steel environmental surfaces.

Methods: Using unpaired samples, each method was evaluated against 30 stainless steel test portions: 20 test portions inoculated at a low inoculation level of ~50 CFU/4"x4"; 5 test portions inoculated at a high inoculation level of ~100 CFU/4"x4"; and 5 uninoculated control test portions. After sample enrichment and incubation, test portions were assayed by the instructions for use (IFU) from each of the five rapid methods. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG 8.09.

Results: Results for each assay were compared to the MLG method by a POD statistical analysis. No significant differences were observed between the new method and the reference method.

Significance: This new method demonstrated reliability as an easy-to-use, rapid method for the detection of *Listeria* species on environmental surfaces.

P2-98 A Comparative Evaluation of the Veriflow *Listeria* Species (LS) Method for the Detection of *Listeria* Species in Select Foods and Environmental Surfaces

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Introduction: The Invisible Sentinel Veriflow *Listeria Species (LS)* method utilizes a PCR-based detection method coupled with a rapid vertical flow-based assay to rapidly and accurately detect *Listeria* species in food products and on environmental surfaces in only 24 hours of non-specialized incubation. The new method eliminates the need for cumbersome sample preparation steps, gel electrophoresis or fluorophore based detection of target amplifications. This new method provides the specificity and sensitivity of PCR-based amplification in a cost-efficient and easy-to-use format.

Purpose: The purpose of this independent evaluation was to compare the new method to the USDA/FSIS-MLG 8.08 for ready-to-eat (RTE) meats and environmental surfaces and to conduct Inclusivity and Exclusivity testing as part of the AOAC Research Institute™ validation process.

Methods: The method comparison analyzed 4 environmental surfaces (stainless steel, ceramic tile, sealed concrete and plastic) and 2 RTE meats (deli turkey (125 g) and hot dogs (25 g)). Each matrix was inoculated with a different strain of *Listeria* species. After sample enrichment and incubation,

samples were lysed and the target DNA was amplified. The amplified DNA was mixed with a proprietary buffer, transferred to a Veriflow cassette and results were obtained within 3 minutes. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG. For the inclusivity and exclusivity evaluation, 50 *Listeria species* isolates and 30 closely related non-*Listeria* species isolates were evaluated.

Results: A POD statistical analysis indicated no significant differences observed between the new method and the reference method for all matrices. For inclusivity, 50 out of 50 strains of *Listeria* species were correctly identified. All 30 exclusivity organisms were correctly excluded.

Significance: This new method demonstrated high sensitivity and specificity as an easy to use rapid method for the detection of *Listeria* species in select foods and environmental surfaces.

P2-99 A Comparative Evaluation of the TEMPO BC for the Detection of *Bacillus cereus* Group in Food Products and on Environmental Surfaces

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Introduction: *Bacillus* cereus is a Gram positive, aerobic spore-forming bacterium that when reaching levels >10^6 cells/g can lead to food poisoning. *B. cereus* has been isolated from prepared foods that have been improperly stored, as well as being incriminated in past foodborne outbreaks. The TEMPO BC assay is an automated enumeration method, that allows for the quantification of *Bacillus cereus* group in as little as 22 hours. The method works by combining samples with a selective culture medium containing a substrate which, when reduced, emits a signal detected by the TEMPO reader. The inoculated medium is introduced into a test card containing 48 wells across 3 different volumes. Depending on the number and size of positive wells, the TEMPO system deduces the number of *Bacillus cereus* group present in the sample according to a calculation based on the Most Probable Number (MPN) method.

Purpose: The purpose of this extension was to compare the new method to the FDA/BAM Chapter 14 for the enumeration of *Bacillus cereus* in a broad range of foods.

Methods: In this evaluation, 129 different food products or ingredients were enumerated for the presence of *B. cereus.* More than half of the samples were evaluated for the presence of naturally occurring *B. cereus.* Test portions were diluted 1:10 using Butterfield's phosphate buffer and analyzed by the TEMPO BC or FDA/BAM method. Test portions tested by the new method were analyzed at 22.5 ± 0.5 hours. The results of the TEMPO enumeration were verified by plating the test portions onto MYP and BACARA test media.

Results: Results for the evaluation show that the new method was statistically comparable to the FDA/BAM reference method.

Significance: The new method demonstrated provided reliable, rapid and accurate results for the quantitation of *Bacillus cereus* group in a broad range of foods.

P2-100 Comparison of Thermal Resistance Parameter Measurement of *Salmonella* in Skim Milk between Isothermal and Non-isothermal Heat Inactivation Methods

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Developing Scientist Competition

Introduction: Isothermal heat inactivation is a traditional method to measure thermal death time parameters, D_T and z. Because of the number of samples required, this method is time and labor intensive. This study explored the measurement of D_T and z-values using a non-isothermal method which promises to significantly reduce the number of samples required for their calculation.

Purpose: To develop a non-isothermal DSC method in measuring D_T and z-values.

Methods: The non-isothermal heat treatment used a differential scanning colorimeter (DSC) with a constant heating rate of 5°C/min, and the isothermal treatment a water bath using capillary tubes. In the DSC, *Salmonella* Senftenberg in skim milk was heated from 25 to target temperatures and in capillary tubes, it was heated at a constant 65.9, 67, and 68°C for various lengths of time. Under non-isothermal treatment, D_T and z values were calculated by fitting the data to the following equation: $\log(N_t/N)=(z/(K*D_{Tref}*\ln 10))*(10\text{^}((T-T_{ref})/Z))$. Under isothermal conditions, the standard log linear approach was used to calculate these values. All experiments were conducted with three or more replicates.

Results: Under isothermal heat treatment, the D_T values at 65.9, 67, and 68°C were 0.37, 0.2 and 0.14 min, respectively, and the derived z-value was 4.9°C. Under non-isothermal treatment, it was found that heat transfer uniformly affected results requiring a correction to the target temperature. This was based on the energy flow profile (q=Cp*M*(target temperature increase-lapse)) and a temperature correction of 2.82°C was determined. When corrected to the skim milk temperatures of 66, 67, and 68°C, the respective D_T- values were 0.43, 0.23, and 0.13 min, and the z-value is 3.8°C.

Significance: Incorporating the difference between the sample and programmed temperature, this non-isothermal approach aligns perfectly with the capillary tube method in measuring D_T and z-values, and reduces revolutionarily the time and effort involved in measurement.

P2-101 Validation of iQ-Check Real-time PCR Kit for Detection of *Salmonella* spp. from Selected Foods with a 375 g Sample Size

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Introduction: *Salmonella*-contaminated food can be a cause of human illness. Testing for the presence of the bacteria in food is an important practice. The use of molecular methods, such as real-time PCR, can greatly decrease time to results with increased sensitivity.

Purpose: It has become common industry practice to test large composite samples of food for pathogens, especially with a 375g size. The iQ-Check kit is a real-time PCR method utilizing a novel fluorescent double stranded DNA hybridization probe for detection of *Salmonella*. A modification of the current AOAC Performance Tested Method (#010803) has been completed using a 375g sample size.

Methods: In an independent method comparison study, the test method was compared to the USDA FSIS Microbiology Laboratory Guidebook reference method for the analysis of raw ground chicken and deli ham and to the US FDA Bacteriological Analytical Manual reference method for the analysis of dry dog food and non-fat dry milk powder. Thirty samples were tested for each matrix, 5 uninoculated, 20 low level fractional positive and 5 high level. A food-to-media dilution ratio of 1:4 was tested to decrease the volume of liquid broth needed with this large sample size. The iQ-Check Prep automation system was utilized for DNA extraction and PCR preparation.

Results: The proportion of positive analytical outcomes for each matrix at a given level was calculated by using POD statistics. Results demonstrated there was no significant difference in the performance of the test method when compared to the appropriate reference method.

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Significance: The method presented provides users a validated method for detection of *Salmonella* from a large 375g sample size with the proven accuracy of real-time PCR. The use of the automation system allowed users to tend to other lab duties while the instrument processed the samples. It also provided sample traceability.

P2-102 A Comparative Evaluation of Romer Lab's RapidChek SELECT *Salmonella* Test System against a Variety of Rapid Methods for the Detection of *Salmonella*

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Introduction: Current *Salmonella* cultural methods are often time consuming and can take up to 5 days to obtain a negative result. There is a strong need in the food industry to have methods that are rapid and accurate, yet simple to use. The Romer Lab's RapidChek SELECT *Salmonella* Test System employs a proprietary media supplemented with phage to accurately detect foodborne pathogens in food and environmental surfaces. The new technology utilizes phage as a selective agent during enrichment, and with innovative immuno-strips, improves sensitivity and specificity while decreasing the presence of cross reactive and competitive bacteria, reducing the chance for false positive results in high burden samples.

Purpose: The purpose of this evaluation was to evaluate the new method, along with two commercially available enzyme immunoassays and three PCR assays, for the detection of *Salmonella* on stainless steel environmental surfaces.

Methods: Using an unpaired study design, each method was evaluated against 30 stainless steel test portions: 20 test portions inoculated at a low inoculation level of ~100 CFU/4"x4"; 5 test portions inoculated at a high inoculation level of ~400 CFU/4"x4"; and 5 uninoculated control test portions. After sample enrichment and incubation, test portions were assayed by following the instructions for use (IFU) for each of the six methods. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG 4.06.

Results: Results for each assay were compared to the MLG method by a POD statistical analysis. No significant differences were observed between the new method and the reference method.

Significance: This new method demonstrated reliability as an alternative, rapid method for the detection of *Salmonella* species on environmental surfaces.

P2-103 Concentration of the Foodborne Pathogen *Salmonella* from Complex Spice Samples for Molecular Diagnostics Using the InnovaPrep Concentrating Pipette

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Introduction: The Food and Drug Administration has recently reported that approximately 7% of imported spices are contaminated with the foodborne pathogen, *Salmonella*. Detection and identification of pathogens by molecular diagnostics can provide accurate results in significantly shorter time periods, but further improvement in sensitivity is attained through concentration of the target organism(s).

Purpose: The purpose of this study was to evaluate and validate the performance of the InnovaPrep Concentrating Pipette as a tool for the concentration of *Salmonella* from complex spice samples.

Methods: Ten common spices were collected from bulk spice markets. These samples were cultured for 24 hours in 50 ml of Buffered Peptone Water as described in the USDA *Salmonella* protocol (sample to media ratio: 1/100). One ml samples were collected from the cultures (n = 3) for DNA extraction using the SureTec sample preparation chemistry and protocol. Cultures that tested positive (n = 4; turmeric, thyme, black pepper, and oregano) where processed using the Concentrating Pipette. The remaining culture media (47 mls) was processed with a 0.4 micron polycarbonate Concentrating Pipette Tip using the concentrator and was eluted with 200 µl wet foam elution buffer and collected into a sterile tube. This resulted in a 235:1 concentration of the sample. Ten microliters of the concentrated sample were then processed for PCR using the SureTec protocol as described above. Following cell lysis, the samples were amplified using the SureTec *Salmonella* PCR reagents.

Results: Amplification showed a significant improvement in crossing threshold cycle number to determine a positive result. *Salmonella*-positive spice samples (n = 4) showed a mean improvement of 15 cycles to achieve a positive result (23 cycles).

Significance: This study demonstrates the utility of the InnovaPrep Concentrating Pipette for the cleanup and concentration of *Salmonella* from complex spice samples.

P2-104 Validation of a Single 18 h Selective Enrichment and Chromogenic Media Detection of *Salmonella* spp. from Selected Foods

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Introduction: *Salmonella* had been one of the most frequently reported causes of bacterial foodborne illness in the United States. Common foods associated with *Salmonella* infection include chicken, eggs, cantaloupe and peanut butter. The use of selective broth combined with a chromogenic agar with results based on color change enzymatic reactions increases ease of use and reduces the time to result.

Purpose: RAPID'*Salmonella* is a chromogenic media for detection of *Salmonella* spp. in food based on two simultaneous enzymatic activities, identification of *Salmonella* spp. by C8-esterase activity and differentiation from other enterobacteria by β-glucosidase activity. The media was granted AOAC Performance Tested Method Status (# 050701) in May of 2007. A modification of the method has been tested using a single shortened (18h) enrichment time in buffered peptone water supplemented with a mix of selective agents.

Methods: In a method comparison study, the test method was compared to the US Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (USDA MLG) reference method for the analysis of chicken raw ground chicken and to the US Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) reference method for the analysis of eggs, cantaloupe and peanut butter. One hundred *Salmonella* strains were tested in an inclusivity study and 31 non-target organisms were tested in an exclusivity study.

Results: Method comparison results demonstrated there was no significant difference in the performance of the RAPID'*Salmonella* method when compared to the appropriate reference method. Inclusivity and exclusivity rates were 97% and 90%, respectively.

Significance: The method presented greatly shortens the time to a *Salmonella* result by eliminating the need for a secondary enrichment step. By utilizing a chromogenic color change reaction to identify *Salmonella*, colonies can be selected more easily and with greater confidence so action can be taken on potentially contaminated food.

P2-105 Internal Validation of a Process for Wet Pooling of Five Environmental Samples and Subsequent Detection of *Listeria* spp. by Real-time PCR

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Introduction: Pooling, also referred to as wet pooling, is the combining of multiple post-enriched samples into one sample to run on a rapid detection method. The advantage of sample pooling is that it can significantly reduce the costs per test in a sampling program.

Purpose: When samples are pooled, the detection method used must be sensitive enough to detect one presumptive positive sample that has potentially been diluted by four negative samples. The objective of this study was to test the effectiveness of iQ-Check *Listeria* spp. at detecting various strains of *Listeria* in five-sample post-enriched pooled environmental sponge samples and determine the fractional positive detection limit of the kit with diluted pooled samples.

Methods: *Listeria monocytogenes, L. innocua, L. welshimeri and L. ivanovii* were cultured and inoculated each into one individual environmental sponge at a target level of <10 cells. Three competitor organisms (*Staphylococcus aureus, Enterococcus gallinerum* and *Leuconostoc pseudomesenteroides*) were cultured and inoculated into all five environmental sponges at a target level of >50 cells. After an overnight enrichment, one *Listeria* inoculated sample was pooled with four competitor inoculated samples. In addition, the *L. monocytogenes* sponge pool was diluted to verify the detection limit of the method.

Results: All inoculated samples were positive as were all wet pooled samples. While there was a 2-3 Cq difference between the individual sample run on its own and the pooled sample, all samples were still positive above the threshold. The *L. monocytogenes* dilution series showed the fractional positive level of the assay to be between 10^2–10^3 CFU/ml.

Significance: This study shows that the sensitivity of the iQ-Check *Listeri*a spp. kit is not compromised when wet pooling was performed. Pooling samples using a method with a sensitive enough detection limit to withstand the dilution provides an economical way to test for *Listeria*.

P2-106 Performance Assessment of the Thermo Scientific™ SureTect™ *Salmonella* species Real-time PCR Assay According to the ISO 16140 Standard for *Salmonella* spp. Detection in Food and Pet Food

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Introduction: The Thermo Scientific™ SureTect™ Salmonella species Real-Time PCR Assay is a new detection method based on real-time PCR. The oligonucleotides target unique DNA sequences found only in the target micro-organism and use PCR technology to amplify and detect them. If present, the target DNA is amplified and the increasing fluorescent signal is detected by the Thermo Scientific PikoReal Real-Time PCR instrument and interpreted by the Thermo Scientific SureTect Software.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 6579 standard, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The alternative method includes a single enrichment step. Different protocols are available depending on the tested food matrices. After DNA extraction, PCR is run on the PikoReal instrument.

Results: 442 samples were analyzed to determine the relative accuracy, sensitivity and specificity of the alternative method. The results demonstrate equivalent performance between the alternative method and ISO 6579. Depending on the tested (matrix/strain) pairs, the relative detection limits of the alternative method vary from 0.2 to 1.5 CFU/25 g, those of the ISO standard vary from 0.2 to 1.1 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by testing 51 target strains and 30 non-target strains. The alternative method was also evaluated in a ring trial involving 10 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the alternative method precision is equivalent to ISO 6579.

Significance: The SureTect™ Salmonella species Real-Time PCR Assay is a reliable method for *Salmonella spp.* detection in food and pet food, and offers important economic savings by reducing time to result and handling time.

P2-107 Performance Assessment of the Thermo Scientific SureTec *Listeria monocytogenes* Real-time PCR Assay – According to the ISO 16140 Standard for *Listeria monocytogenes* Detection in Food and Environmental Samples

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Introduction: The Thermo Scientific™ SureTect™ *Listeria monocytogenes* Real-Time PCR Assay is a new detection method based on real-time PCR. The oligonucleotides target unique DNA sequences found only in the target microorganism and use PCR technology to amplify and detect them. If present, the target DNA is amplified and the increasing fluorescent signal is detected by the Thermo Scientific PikoReal Real-Time PCR instrument and interpreted by the Thermo Scientific SureTect™ Software.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 11290-1 standard, as part of the NF Validation approval process and according to the ISO 16140 standard

Methods: The alternative method includes a single step enrichment in 24 LEB supplemented with both 24 LEB Buffer and selective supplements for 22-26h at 37°C. After DNA extraction, PCR is run performed using the PikoReal instrument.

Results: 339 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity. The results demonstrate equivalent performance between the alternative and ISO 11290-1 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the alternative method vary from 0.2 to 1.2 CFU/25 g, those of the ISO standard vary from 0.2 to 1.1 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by testing 50 target strains and 30 non target strains. The alternative method was also evaluated in a ring trial involving 10 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the alternative method precision is equivalent to the ISO 11290-1 reference method.

Significance: The SureTect™ Listeria monocytogenes Real-Time PCR Assay is a reliable method for *Listeria monocytogenes* detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

P2-108 Performances Assessment of the 3M Molecular Detection Assay *Eschericha coli* O157 (including H7) Kit According to the ISO 16140 Standard for *E. coli* O157 Detection in Raw Beef Meats, Raw Dairy Products, Raw Fruits and Vegetables

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Introduction: The 3M™ Molecular Detection Assay *Escherichia coli* O157 (including H7) kit uses isothermal amplification of specific DNA target sequences. The amplification is detected by bioluminescence.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 16654 standard, as part of the NF VALIDATION approval process and according to the ISO 16140 standard.

Methods: The 3M™ Molecular Detection Assay *E. coli* O157 (including H7) test protocol includes a single enrichment step in pre-warmed Buffered Peptone Water (ISO) at 41.5°C. After lysis, DNA amplification is performed in the 3M™ Molecular Detection Instrument.

Results: 185 samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the 3M Molecular Detection Assay *E. coli* O157 (including H7) and the ISO 16654 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the 3M Molecular Detection Assay *E. coli* O157 (including H7) method vary from 0.2 to 1.0 CFU/25 g, those of the ISO standard vary from 0.2 to 1.3 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by tested 50 target strains and 31 non target strains. The 3M Molecular Detection Assay *E. coli* O157 (including H7) method was also evaluated in a ring trial involving 12 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the 3M Molecular Detection Assay *E. coli* O157 (including H7) method precision is equivalent to the ISO 16654 standard.

Significance: The alternative method is a reliable method for *Escherichia coli* O157 (including H7) detection in raw beef meats, raw dairy products, raw fruits and vegetables, and offers important economic savings by reducing time to result and handling time.

P2-109 ISO 16140 Certification of a New Alternative to Detect *Cronobacter* spp. in Infant Formula and Environmental Samples

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Introduction: The iQ-Check™ *Cronobacter* method is based on the real PCR principle for *Cronobacter* spp. detection in infant formula and environmental samples. An ISO 16140 method comparison study was conducted, by analyzing 171 samples in the relative accuracy, sensitivity and specificity part and showing equivalent performances between the alternative method and the ISO/TS 22964 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the Real-Time PCR method spp method vary from 0.5 and 1.6 CFU/25g, those of the ISO standard from 0.5 to 1.5 CFU/25g. The selectivity and specificity of the alternative method was assessed by testing 52 target strains and 31 non-target strains.

Purpose: An independent inter-laboratory study was conducted at ADRIA, to compare the alternative method precision to the ISO/TS 22964 one, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The iQ-Check™ *Cronobacter* spp. 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, the 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: The alternative method was evaluated in a ring trial involving 13 laboratories. Probiotic infant formula was contaminated with the wild *C. sakazakii*Ad 940 strain. 8 blank samples, 8 samples contaminated at a fractional recovery level (0.8 cells/g) and 8 highly contaminated samples (20.6 cells/g) were sent to each collaborator. At the fractional recovery and high inoculation levels, the sensitivity values of the standard method were, respectively, 52% and 100%, those of the alternative method 55% and 99%.

Significance: This ISO 16140 study clearly shows that the iQ-Check™ *Cronobacter* method is a reliable alternative method for *Cronobacter* spp. detection in infant formula and environmental samples, offering important economic savings by reducing time to result and handling time.

P2-110 Performances Assessment of the 3M Molecular Detection Assay *Salmonella* Kit According to the ISO 16140 Standard for *Salmonella* spp. Detection in Food Products and Environmental Samples

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Introduction: The 3M™ Molecular Detection Assay *Salmonella* kit uses isothermal amplification of specific DNA target sequences. The amplification is detected by bioluminescence.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 6579 standard, as part of the NF VALIDATION approval process and according to the ISO 16140 standard.

Methods: The 3M™ Molecular Detection Assay *Salmonella* test protocol includes a single enrichment step in Buffered Peptone Water (ISO). Two incubation temperatures are validated depending on the tested food categories (37°C and 41.5°C). After lysis, DNA amplification is performed in the 3M™Molecular Detection Instrument.

Results: 325 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the 3M™ Molecular Detection Assay *Salmonella* and the ISO 6579 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the 3M Molecular Detection Assay *Salmonella* method vary from 0.3 to 2.0 CFU/25 g, those of the ISO standard vary from 0.3 to 1.8 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by tested 50 target strains and 30 non target strains. The 3M™ Molecular Detection Assay *Salmonella* method was also evaluated in a ring trial involving 15 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the 3M™ Molecular Detection Assay *Salmonella* method precision is equivalent to the ISO 6579 standard.

Significance: The alternative method is a reliable method for *Salmonella spp.* detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

P2-111 Comparative Evaluation of the VIDAS® UP *Listeria* (LPT) for the Detection of *Listeria* spp. in RTE Meat and Environmental Surfaces

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Introduction: VIDAS® UP *Listeria* (LPT) is a phage-ligand based immunoassay for the rapid detection of *Listeria* spp. in food and environmental samples, using a single enrichment step in proprietary LPT broth.

Purpose: The phage-ligand based immunoassay was compared to Assurance GDS® for *Listeria* spp. (PCR1) and BAX® Genus *Listeria* 24E (PCR2) for the detection of *Listeria* in environmental samples and ready-to-eat deli meat.

Methods: Environmental samples (stainless steel, plastic, concrete) and deli ham were inoculated with *Listeria* spp. (30 replicate samples; 0.2-2 CFU/test portion) and 5 uninoculated controls. All test portions were enriched and analyzed for the detection of *Listeria* spp. according to manufacturer recommended procedures for the alternative methods and the USDA-FSIS-MLG reference method.

Results: For the combined 90 spiked environmental surfaces, the LPT method detected 42 *Listeria* positive samples compared to 34 for GDS and 36 for BAX. In the deli-ham, the phage-ligand based immunoassay *Listeria* method detected 19 *Listeria* positive samples compared to 13 with PCR1 and 10 for PCR2 out of the 30 inoculated samples. Both POD and chi-squared analysis of these unpaired factional positive samples for the three methods show no statistical difference in recovery.

Significance: The phage-ligand based immunoassay method with single-step enrichment and next day automated detection was statistically equivalent to the PCR1 and PCR2 molecular methods and reference method for detecting *Listeria* spp. in the environmental and food matrices tested.

P2-112 Field Study Demonstrates High Concordance between the Roka Bioscience Atlas® *Listeria* Detection Assay and USDA-FSIS MLG Protocol 8.09

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Introduction: Routine verification testing for *Listeria* species is commonly included in an establishment's *Listeria* Control Program. Novel molecular detection assays may improve testing efficiency, shortening the time before actions are taken regarding the presence of these *Listeria monocytogenes* indicators in the processing environment.

Purpose: This field study was conducted to determine the concordance of the Roka Bioscience Atlas Listeria Detection Assay (LDA) with a validated USDA-FSIS MLG protocol for *L. monocytogenes* using environmental sponge samples collected from two meat processing facilities.

Methods: Duplicate sponge samples were collected mid-shift from 101 environmental sites in the two facilities. One whole sponge was subjected to enrichment and detection via the LDA Protocol while the other was enriched and cultured according to the USDA-FSIS MLG Protocol 8.09 with modifications. Concordance between the two methods is reported as the percentage of total samples with identical outcomes. For discrepant LDA detection and MLG culture results, an aliquot of LDA enrichment was subjected to MLG culture procedures to assess agreement between the detection result and presence of viable listeriae in the enrichment.

Results: Concordance between the two methods was high (94.1%) with 11.9% and 82.2% of samples having LDA(+)/MLG(+) or LDA(-)/MLG(-) outcomes, respectively. Discrepancies arose in 6.0% of samples with 5.0% reported as LDA(-)/MLG(+), and 1.0% as LDA(+)/MLG(-). Culturing the LDA enrichment for LDA(-)/MLG(+) samples yielded no growth on plates, revealing that these LDA detection results were accurate given the absence of viable listeriae. Culturing the LDA enrichment of the lone LDA(+)/MLG(-) sample also yielded no growth though the LDA detection yielded a second positive result when repeated for verification.

Significance: The high concordance between the two methods demonstrates that detection via the LDA protocol is comparable to the USDA-FSIS MLG protocol for *L. monocytogenes*. Discrepancies that arose can likely be attributed to inconsistencies in sampling or enrichment.

P2-113 Evaluation of a Real-time PCR Assay for the Detection of Genus *Listeria*

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Introduction: Rapid screening methods for *Listeria* species can assess potential risk of *Listeria monocytogenes* contamination of foods and the efficacy of environmental cleaning and disinfection. PCR screening methods provide sensitivity, specificity, ease of use and faster time-to-result relative to standard cultural methods.

Purpose: This study evaluates the new DuPont™ BAX® System Real-Time PCR Assay for Genus *Listeria*, which utilizes shorter lysis protocol and faster processing times than current BAX® System assays for *Listeria*. Assay sensitivity, inclusivity and exclusivity were assessed in pure culture and in food and environmental surface enrichments relative to standard methods.

Methods: Inclusivity was evaluated by processing 77 different *Listeria* strains serially diluted to approximately 10^5 CFU/ml, which is 1-log above the sensitivity limit of the assay. Exclusivity was tested with pure cultures of 75 different non-*Listeria* strains at ≥10^8 CFU/ml. For each of six different food and environmental surfaces, 5 high-spike, 20 low-spike and 5 un-spiked samples were tested with the PCR method after enrichment in proprietary 24LEB Complete, and results were compared to independent spiked samples enriched in BLEB or UVM as appropriate.

Results: For the strains tested, the assay demonstrated 100% inclusivity and 100% exclusivity. No statistical difference was observed in the number of positive spiked food and environmental surface enrichments detected by PCR relative to the standard enrichment and confirmation protocols.

Significance: This new assay allows for a more rapid time-to-result for the testing of food and environmental samples than standard methods, while maintaining the simplicity, accuracy and reliability of the BAX® System.

P2-114 Evaluation of Test-kits for the Detection of *Escherichia coli* O157 in Raw Meats and Cattle Faeces

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Introduction: Investigation of foods implicated in disease outbreaks indicates that the infectious dose for *E. coli* O157:H7 is fewer than 50 cells. Since *E. c*oli O157 may be present in food and environmental samples in only small numbers and the infectious dose is low, sensitive and rapid detection methods are needed to ensure a safe supply of foods.

Purpose: The objectives of this study were to determine the relative sensitivity and specificity of VIDAS-ICE, VIDAS-UP, real-time PCR, and immunomagnetic *E. coli* O157 detection methods.

Poster

Methods: *E. coli* O157 detection limits in artificially contaminated beef and cattle faeces samples enriched in either modified tryptone soya broth with novobiocin (mTSB+n) or buffered peptone water (BPW) were determined for Dynabeads anti *E. coli* O157 immunomagnetic beads, VIDAS-ICE, VIDAS-UP and real-time PCR (GeneDisc and LightCycler) systems.

Results: Dynabeads anti- *E. coli* O157 immunomagnetic separation (IMS) and the GeneDisc cycler were the most sensitive methods, and could detect an initial 1 CFU in beef samples after 6h of incubation in mTSB+n or BPW. The VIDAS-UP method could detect an initial 10 CFU, while VIDAS-ICE and the LightCycler methods could only detect an initial 100 CFU. Higher detection rates were achieved with 18 hour incubations, where an initial 1 CFU could be detected with all five methods. For cattle faeces enrichments, Dynabeads anti *E. coli* O157 IMS could detect an initial 1 CFU after a 6h incubation in mTSB+n, while the VIDAS-UP and VIDAS-ICE methods could detect an initial 10 CFU and both PCR methods could only detect an initial 100 CFU. Detection rates were lower in BPW, compared to mTSB+n, with thresholds of 100 CFU for VIDAS-ICE, VIDAS-UP and GeneDisc methods, and >100 CFU for the LightCycler method.

Significance: GeneDisc, and VIDAS-UP *E. coli* O157 detection methods can be used as rapid screening methods before final confirmation on selective agar as the two methods showed high sensitivity and specificity for *E. coli* O157 which is complemented by high levels of automation and relative ease of use.

P2-115 A Thermal Resistance Study of STEC in Low-moisture Foods with the Use of Differential Scanning Calorimeter (DSC)

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Introduction: According to CDC's Foodborne Disease Outbreak Surveillance System database, from year 2007~2011, there have been eight outbreaks associated with low-moisture foods including nuts, cheese, cookie dough, and wheat snack foods involving Shiga toxin-producing *Escherichia coli* O157:H7 (STEC), which led to over 318 cases of foodborne illness. Yet, insufficient data on STEC thermal inactivation has been obtained in low-moisture foods. In this study, a differential scanning calorimeter is used to measure STEC inactivation kinetic parameters in low moisture environments.

Purpose: The objective of this study was to use a differential scanning calorimeter to measure D- and z-values, and therefore determine the microbial thermal resistance, of STEC.

Methods: Six strains of outbreak related *E. coli* were individually grown on tryptic soy agar with yeast extract (TSAYE). The cells were harvested and inoculated into a moist buffer solution, simple model low-moisture matrix, and low-moisture foods (i.e., flour and peanut butter). Samples were individually heated using a differential scanning calorimeter (DSC). The DSC was able to achieve a reproducible and accurate thermal environment. Following heat treatment, microbial survivors were enumerated via plate count.

Results: The six strains showed greater thermal resistance levels in corn syrup and peanut butter compared with buffer solution ($P < 0.05$). At the same processing duration, approximately 95°C was needed to reduce the outbreak strains of STEC in peanut butter by 5-log CFU/ml, whereas 85°C and 75°C were needed for corn syrup and buffer solution, respectively. $D_{65°C}$ ranged from 0.26-0.92 min for the strains tested in buffer. Those same strains exhibited a 10-100 times increase in resistance in corn syrup ($D_{65°C}$= 2.6-108.6 min).

Significance: The measurement of the increased heat resistance of STEC in low moisture foods will improve science-based risk prevention by ensuring process lethality in these types of foods.

P2-116 Fluorescent Ca^{2+} Indicator-based B Cells Biosensor for Rapid Detection of *Escherichia coli* O157:H7 in Foods

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Introduction: *Escherichia coli* O157:H7 is one of the leading bacterial pathogens causing foodborne illness, infecting an estimated 73,000 people in the US each year and having an infective dose as low as 10 cells. A rapid, sensitive, and specific detection method for *E. coli* O157:H7 is continuing needed.

Purpose: The objective of the present study was to develop a fluorescent Ca^{2+} indicator based B cells biosensor with better combined speed and sensitivity than current methods for rapid detection of *E. coli* O157:H7 in foods.

Methods: The B cell membrane was firstly engineered with antibodies specifically against *E. coli* O157:H7. Then, a fluorescent Ca^{2+} indicator (Fura-2) was transferred into the B cell, which had an emission wavelength at 510 nm. When the target pathogen was attached to its specific antibodies on B cell surface, it produced a signal, and the signaling pathways were activated, resulting in the release of Ca^{2+} within seconds. The elevated intracellular Ca^{2+}concentration activated Fura-2 to report the fluorescence signal change and indicated the presence of target pathogen.

Results: The developed B cells biosensor was able to detect *E. coli* O157:H7 at the concentration as low as 70 cells in a sample with a volume of 100 µl and a detection range from 10^2 to 10^8 CFU ml^{-1} was obtained. The total detection time from sampling to detection was within 30 min. The attachment of target *E. coli* O157:H7 on the surface of B cells were further confirmed by SEM (Scanning electron microscope) images.

Significance: The outcome of this study will make the detection of *E. coli* O157:H7 easier, faster, more sensitive and more suited for the ongoing transition from fundamental analytical science to the early diagnosis and detection of pathogens.

P2-117 Applicability of the QIAxcel System and a Multiplex PCR for Shiga Toxin-producing *Escherichia coli* Detection

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Introduction: The major STEC serogroups that cause human infections are O157, O26, O103, O111, O121, O45, and O145 (top-7). Four virulence factors: *stx*1*, stx*2*, eae, ehx*A are commonly associated with more severe forms of human infections. The organisms are reside and propagated in the gastrointestinal tract in cattle and are shed in feces, serving as a major source of food and water contamination. An 11-gene multiplex PCR was established to detect the O-antigens of the "top-7" STECs and the 4 virulence factors with manual data interpretation.

Purpose: To evaluate automatic data interpretations by the Peak-Calling function in the QIAxcel software, and compare with manual interpretation results on amplicons generated by the 11-gene multiplex PCR.

Methods: A total of 185 enriched cattle fecal swab pools (5 per pool) were amplified for 11-genes described above. PCR products were directly run on the QIAxcel without further manipulation. Positive amplifications of each of the 11 genes for each sample were manually interpreted. The results were then compared with data generated automatically by the Peak-Calling method.

Results: Most samples (86.5%) were positive for at least one of the seven O-types, and two virulence genes. Positive bands for individual sample ranged from 2-9 (some samples were positive for more than one O-types), with average of 5 bands. The total number of bands in these 185 samples was 931. Samples were 61.6%, 28.1% and 19.5% positive for O157, O26 and O103 *E. coli* serogroups, respectively. Bands that manually identified were 98.9% the same as those identified by Peak-Calling interpretations.

Significance: From the 185 samples tested, Peak-Calling method identified the same numbers and same sizes of bands to that generated by manual interpretations. Applying the Peak-Calling method post an 11-gene multiplex PCR will significantly save personnel time. The method will also reduce potential interpretation errors generated by manual interpretations.

P2-118 Comparing Apples to Apples: Validating an Elution-based Defined Substrate Method to Enumerate *Escherichia coli* on Tree Fruit

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Introduction: Agricultural water, in particular irrigation and cooling water, has been identified as a potential route for introduction of microbial contamination to apples. However, a full understanding of the degree to which microbial contamination is introduced to fruit surfaces is limited by a lack of simple, efficient, and accurate methods to quantify microbial contamination on tree fruit. This study investigates a quick and easy-to-perform assay that incorporates surface elution and defined substrate technology to quantify *E. coli* on apples.

Purpose: This study aims to evaluate the efficacy of a surface elution method for the enumeration of *E. coli* on apples, utilizing IDEXX Colilert®-18 and QuantiTtray®/2000.

Methods: Unwaxed apple samples were inoculated with *E. coli* at low, medium, and high seeding levels, each differing by an order of magnitude. Inoculated apples were massaged in a Whirl-Pak® with 100ml of PBS for 5 minutes. The eluate was mixed with Colilert®-18 substrate and sealed in a QuantiTray®/2000. After incubation at 37°C for 18 hours, an MPN for *E. coli* was generated for each sample based on the number of positive wells (those that fluoresced under UV light).

Results: Using the surface elution method, the average recovery rate was 11.5% at the lowest inoculation level (~10^2 *E. coli* per sample; n = 16). At the medium inoculation level (~10^3 *E. coli* per sample), the average recovery was 11.2% (n = 16). Samples in the highest seeding level (~10^4 *E. coli* per sample) had an average recovery of 4.1% (n = 10). Apple samples ranged in weight from 112.1g to 198.5g.

Significance: The *E. coli* enumeration method evaluated in this study will aid researchers in conducting quantitative microbial risk assessment studies on apple handling and processing. Future studies utilizing this method may inform food safety policies to minimize the risk of apple microbial contamination.

P2-119 FDA-ECID: A Novel Microarray Representing the PanGenome of *Escherichia coli*: A Tool for Molecular Epidemiology, Molecular Serotyping, and Phylogeny

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Introduction: Illnesses associated with the consumption of foods contaminated with pathogenic *Escherichia coli* result in thousands of hospitalizations and hundreds of deaths annually throughout the world. The ability of these pathogens to rapidly adapt to novel environmental niches necessitates highly parallel analysis methods in order to accurately identify and discriminate individual strains.

Purpose: Here we describe the development and validation of a novel, high density DNA microarray representing all known *E. coli* genes mined from approximately 300 whole genome sequences. The FDA-ECID array has been designed and manufactured using next-generation Affymetrix PEG-GeneAtlas technology. This custom tool is rapid, affordable and high-throughput.

Methods: Using BLASTCLUST and NETCLUST tools, we analyzed 300 whole genome sequences and determined the non-redundant pangenome of the species of *E. coli* to be ~40k unique genes. Each of these ~40k genes is represented as a probe set on our FDA-ECID microarray. Additionally, we have represented each allele from the *fliC*, *wzx*, and *wzy* genes; thereby allowing this microarray the ability to perform molecular serotyping. Using the same 300 genome sequences, we identified ~125k conserved 25-mers each containing a central single nucleotide polymorphism (SNP). Of these, we filtered the most informative 10% that were capable of accurately recapitulating the phylogeny of *E. coli*. Each of the 10k informative SNPs is represented on the FDA-ECID microarray using a SNP-typing probe strategy.

Results: As part of a validation process, we have performed hybridizations in quadruplicate of 4 diverse, well characterized, sequenced reference strains (Sakai, 55989, CFT073, MG1655). These data allowed us to optimize gene-calling and SNP-calling algorithms. We also present the results from our interrogation of a vast collection (>900) of temporally and geographically diverse *E. coli* isolates.

Significance: In summary, the FDA-ECID microarray is a powerful tool for molecular epidemiology, phylogenetic analysis, virulence assessment, molecular serotyping, and exploring the global genomic diversity of *Escherichia coli*.

P2-120 Innovative Phage Protein Ligand Automated Assay to Simplify the Confirmation of TOP 7 EHEC in Raw Beef

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Introduction: The MLG 5B reference method (USDA) recommends an Immuno Magnetic Separation (IMS) for selective concentration of the TOP 7 EHEC serogroups from pre-enriched samples to assist in plate isolation step. However, this technique is cumbersome and difficult to implement routinely. The automated VIDAS® UP *E. coli* Serogroups (ESPT) assay, a specific phage protein ligand assay, has been developed as an alternative to IMS for the simultaneous immuno-concentration of *E. coli* serogroups O26, O45, O103, O111, O121, O145 and O157.

Purpose: To compare the VIDAS ESPT assay with a commercial IMS test for the confirmation of TOP 7 EHEC in 375-g sample size of raw beef.

Methods: 375g artificially inoculated samples, ¼ diluted in a selective pre-warmed Buffered Peptone Water, were enriched for 10 hours at 41.5±1°C and then screened by PCR for the presence of *eae*, *stx* and O groups genes. All presumptive positives were isolated on the chromID®EHEC agar following the VIDAS ESPT and the IMS procedures. Characteristic colonies were further confirmed according to the MLG 5B method.

Results: In this comparative study, 100% of the presumptive PCR positive were confirmed with the new method compared to 86% with the IMS method. Furthermore, in 86% of the cases, the analysis of a single typical colony was sufficient to confirm the presumptive results by using the VIDAS method. For three presumptive positive samples, the analysis of up to ten colonies obtained after IMS did not allow confirmation of the initial PCR results.

Significance: Due to high level of specificity of the recombinant phage protein, the novel assay significantly reduced the background flora on the selective agar and facilitated the recovery of the specific strains. Compared with the traditional IMS, the VIDAS ESPT greatly reduced the number of colonies for subsequent confirmation and improved the overall confirmation rate of the TOP-7-EHEC.

P2-121 Design, Development and Utilization of an *Escherichia coli* Resequencing Microarray: A Tool for Understanding Phylogeny, Genetic Diversity and Molecular Epidemiology

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Introduction: As a commensal, a pathogen, and an emerging pathogen, understanding the evolution and phylogeny of *Escherichia coli* will provide an understanding of its ability to adapt to new environmental niches and to acquire novel virulence and metabolic mechanisms. Over the past three decades, a plethora of molecular assays have been developed to examine its genomic diversity and evolution; including, but not limited to MLEE, MLST, and WGS.

Purpose: The purpose of this study was to describe a MLST-like microarray-based resequencing assay that evolved from an intelligent, rational design strategy.

Methods: Our EC-MLST microarray represents approximately 100 *E. coli* tiles (genes) in a standard resequencing probe design strategy, each approximately 500 bp long and targets the most heterogenic region of each gene, as determined based on gene sequence alignments. Also included are the 30 "standard" loci utilized by Achtman, Whittam, and Pasteur MLST typing schemes and 80 virulence genes that were chosen based on literature searches that revealed their association with particular pathogen types and clinical outcomes.

Results: As part of a validation study, we have determined the accuracy (sequencing error rate) of this resequencing-based assay to be equivalent to Q30. As such, we are able to accurately assess both phylogeny and horizontal gene transfer (recombination). In addition to the validation study, we also present the results of our examination of a vast collection of temporally and geographically diverse isolates of commensal and pathogenic *E. coli* strains. Finally, we present and discuss a data analysis pipeline that allows for automated base calling, curating of reference genome sequence data from Genbank, and comparative genomic-phylogenetic analysis.

Significance: The availability of such a molecular detection method and data analysis pipeline allows for routine use of the EC-MLST array in molecular epidemiological or molecular subtyping scenarios.

P2-122 Genetically Marked Strains of Shiga toxin-producing *Escherichia coli* O157:H7 and Non-O157 for Detection and Modeling

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are among the most important foodborne pathogens in the United States and worldwide. STEC O157:H7 is isolated from about half of all STEC-induced diarrheal disease in North America, while non-O157 STEC account for the remaining isolates. Thus, the USDA Food Safety and Inspection Service has a zero-tolerance policy for regulatory samples that test positive for STEC O157:H7 or six other serogroups of non-O157 STEC (i.e., serogroups O26, O45, O103, O111, O121 and O145).

Purpose: The purpose of this study was to develop a collection of positive-control strains for the detection of *E. coli* O157:H7 and the six USDA-regulated serogroups of non-O157 STEC.

Methods: A unique DNA target sequence and a gene for spectinomycin resistance were integrated into the chromosomes of a strain of O157:H7 and strains of the six non-O157 STEC by allelic exchange to generate positive-control strains for STEC detection. End-point and real-time PCR methods were developed for the specific detection of the control strains. In addition, the strains were tested for their potential use in modeling the growth of STEC in the presence of foodborne background flora by incorporating spectinomycin into selective plating media.

Results: The developed end-point and real-time PCR methods demonstrated excellent inclusivity and exclusivity when tested against 42 strains of STEC O157, at least six strains of each of the USDA-regulated non-O157 STEC, and more than 50 strains of other bacteria representing 29 species and 21 genera. Plating the STEC control strains on modified Rainbow agar containing spectinomycin eliminated the growth of ground beef background flora that otherwise grew on modified Rainbow agar.

Significance: The genetically modified STEC strains are useful as positive control strains for the detection of STEC and will be useful for the modeling the growth of STEC in foods.

P2-123 Studies of the Real-time Rapid Detection of *Staphylococcus aureus* by Isothermal Target and Probe Amplification Assay

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Introduction: In the last decade, Polymerase Chain Reaction (PCR) and Real-time PCR detection methods have been proposed for the detection of foodborne pathogens to replace the time-consuming, culture-based traditional techniques. They are rapid, easy to handle, sensitive, specific and constitute very valuable tools for food safety testing. However, the PCR method still has some disadvantages such as electrophoresis etc. complex operation, requiring expensive heating cycle equipment, and so on. The iTPA technology is simple to operate. It is a novel DNA amplification and fluorescent detection method for a foodborne pathogen by amplifying the target gene and the signal probe simultaneously under isothermal condition with high, specificity and sensitivity.

Purpose: In this study, we designed a set of specific primers and a probe according to the conserved regions of *nuclease (nuc)* gene of *Staphylococcus aureus* and developed a highly sensitive and specific real-time iTPA method for detecting food samples.

Methods: All bacterial strains used in this study were maintained in TSB. Seventy-seven *S. aureus* strains and 51 non-*S. aureus* strains were used to evaluate inclusivity and exclusivity. Sterilized water was used for the negative control. For comparison, a set of PCR reactions was performed using the iTPA outer primers. Specificity tests were repeated 3 times and compared to the traditional method. Real-time iTPA testing in experimentally inoculated various food samples.

Results: The *S. aureus*. *nuc*-based real-time iTPA assay successfully detected *S. aureus* strains while showing negative results for non-*S. aureus* strains, indicating that the *nuc*-based real-time iTPA assay was specific for *S. aureus*. The detection limits of the real-time iTPA assay using serial in *S. aureus* strain were determined and the lowest number of cells detected was 10^2 CFU/ml. The four primers and one FRET probe we designed from five regions of *S. aureus nuc* gene coding sequence that are highly specific to *S. aureus*.

Significance: A novel and rapid real-time DNA detection method has been developed using the real-time isothermal fluorescent equipment which is a much cheaper and simple operation than those of Real-time PCR equipments. We have demonstrated that real-time iTPA can be used to detect *S. aureus* down to 10^2 CFU/ml within 1 hour. The lowest detection limit achieved in this study was a 10^1 CFU per 25 g of food samples. So, *S. aureus* detection kit and iTPA Real time PCR equipment (iGene-*S*) was completed by using this method.

P2-124 Detecting and Tracking Nosocomial Methicillin-resistant *Staphylococcus aureus* Using a Microfluidic SERS Biosensor

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Introduction: Rapid detection and differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) are critical for the early diagnosis of difficult-to-treat nosocomial and community acquired clinical infections and improved epidemiological surveillance.

Purpose: We developed a microfluidics chip coupled with surface enhanced Raman scattering (SERS) spectroscopy (532 nm) "lab-on-a-chip" system to rapidly detect and differentiate methicillin-sensitive *S. aureus* (MSSA) and MRSA using clinical isolates from China and the United States.

Methods: A total of 21 MSSA isolates and 37 MRSA isolates recovered from infected humans were first analyzed by using polymerase chain reaction (PCR) and multilocus sequence typing (MLST). The *mecA* gene, which refers resistant to methicillin, was detected in all the MRSA isolates, and different allelic profiles were identified assigning isolates as either previously identified or novel clones. A total of 17 400 SERS spectra of the 58 *S. aureus* isolates were collected within 3.5h using this optofluidic platform.

Results: Intra- and interlaboratory spectral reproducibility yielded a differentiation index value of 3.43–4.06 and demonstrated the feasibility of using this optofluidic system at different laboratories for bacterial identification. A global SERS-based dendrogram model for MRSA and MSSA identification and differentiation to the strain level was established and cross-validated (Simpson index of diversity of 0.989) and had an average recognition rate of 95% for *S. aureus* isolates associated with a recent outbreak in China. SERS typing correlated well with MLST indicating that it has high sensitivity and selectivity and would be suitable for determining the origin and possible spread of MRSA. A SERS-based partial least-squares regression model could quantify the actual concentration of a specific MRSA isolate in a bacterial mixture at levels from 5% to 100% (regression coefficient, >0.98; residual prediction deviation, >10.05).

Significance: This optofluidic platform has advantages over traditional genotyping for ultrafast, automated, and reliable detection and epidemiological surveillance of bacterial infections.

P2-125 Validation of a Pan Genome DNA Microarray for the Identification and Differentiation of *Cronobacter* spp

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Introduction: *Cronobacter* are opportunistic foodborne pathogens that cause meningitis, necrotizing enterocolitis, and septicemia, particularly among infants and elderly persons. Until recently, the genus was comprised of seven species: *C. sakazakii* (Csak), *C. malonaticus* (Cmal), *C. turicensis* (Ctur), *C. dublinensis* (Cdub), *C. muytjensii* (Cmuy), *C. condimenti* (Ccond), and *C. universalis* (Cuni). All except for Ccond are established human pathogens. In 2013, three nonpathogenic species originally classified as *Enterobacter* spp. (now named *C. helveticus*, *C. pulveris*, and *C. zurichensis*) were included.

Purpose: Validation of the array was done by hybridizing DNA from sequenced strains represented on the array. Also, a study focusing on the interrogation of 122 *Cronobacter* and related strains was conducted to answer whether the microarray could be used as a food safety tool.

Methods: Comparative analysis of whole genome sequences from 17 *Cronobacter* strains led to the design of a custom Affymetrix DNA microarray. The array represents the pan genome for *Cronobacter* and species-specific features and contains 21,432 genes. For each gene, there are 11 probe pairs; each represented by a perfect match and corresponding mismatch probe. Hybridization intensities of these 25-mer probe pairs were used to determine the absence/presence of a gene.

Results: Results suggest that the identity of a *Cronobacter* isolate and its total gene content can be resolved. Furthermore, these results support the divergence of the genus from the most recent common ancestral species into two clusters, one consisting of Cdub-Cmuy and the other comprised of Csak-Cmal-Cuni-Ctur. Ccond was a distant outlier of the two larger clusters. Microarray interrogation of *C. helveticus*, *C. pulveris* and *C. zurichensis* strains support the hypothesis that these organisms taxonomically should not be considered as *Cronobacter*.

Significance: The current study establishes a powerful platform for further functional genomics research of this diverse group, an important prerequisite towards future development of countermeasures against this foodborne pathogen.

P2-126 Evaluation of Molecular Methods for Detection and Genotyping of Norovirus

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Introduction: The detection and identification of virus contaminants are essential for investigation and prevention of foodborne outbreaks. Norovirus is one of the most important causative agents of foodborne gastroenteritis. Due to the lack of a cell culture system, molecular methods have been applied to detect and genotype norovirus.

Purpose: The purpose of this study was to assess the effectiveness and suitability of the different molecular methods for detecting and identifying foodborne pathogens from food and clinical samples.

Methods: We adopted three approaches, RT-qPCR, DNA tiling microarray, and NextGen sequencing (NGS). RT-qPCR was evaluated in detection of human norovirus (NoV), together with extraction control, murine norovirus (MNV), following extraction by the ultracentrifugation method (with PVP) from a variety of foods. Microarray and NGS were applied to detection and genotyping of NoV present in human fecal samples.

Results: While MNV was extracted with a recovery rate ranging from 10% to 40% and detected efficiently by RT-qPCR, none of the foods implicated in the recent noro GII outbreak tested positive for NoV. DNA microarray can detect and genotype norovirus when the sequence of the target virus is more than an 80% match to the probes, the signal intensity directly correlated to the % match. The depth of coverage (> 200X) obtained from NGS of the stool sample is sufficient to generate useful viral sequence contigs leading to de novo assembly and match to published GenBank sequences.

Significance: We demonstrate the applications of real-time RT-qPCR, microarray, and NGS for detection of norovirus in clinical or food samples. These methods have the potential to address the differential needs in surveillance and outbreak investigations.

P2-127 Evaluating Viral Process Controls: Turnip Crinkle Virus and Tulane Virus Demonstrate Similar RNA Extraction Efficiencies to Norovirus Genogroups I and II

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Introduction: Noroviruses are a leading cause of foodborne disease. Historically, noroviruses have been difficult to detect in food and environmental samples, in part due to low concentrations and the lack of a cell culture system. RT-qPCR is a powerful tool for detection of noroviruses; however, processing complex samples in preparation for RT-qPCR often results in low and variable virus recovery efficiencies.

Purpose: This study evaluated several viruses for their utility as process (extraction) controls for human noroviruses during the steps of RNA extraction and RT-qPCR.

Methods: Several viruses, including murine norovirus (MNV), Tulane virus (TV), turnip crinkle virus (TCV), and an F-RNA coliphage (MS2), were compared to a GI.6 and a GII.4 human norovirus relative to recovery efficiency using the BioMérieux Nuclisens easyMAG RNA extraction system and an Invitrogen SuperScript III Platinum One-Step qRT-PCR kit. All viruses were compared at a range of input virus from 2 – 7 log genome equivalent copies (GEC) per 100 µl of DEPC-treated water.

Results: NoV GI.6 and GII.4 had similarly high recoveries (losses of -0.2 ± 0.4 and 0.0 ± 0.3 log GEC, respectively) at all input levels. The virus with the most similar recovery efficiency was TCV, which had losses of 0.0 ± 0.6 log GEC at lower viral inputs ($P > 0.05$) and losses of 0.7 ± 0.7 log GEC at higher inputs. MNV had the lowest recovery efficiency, with losses of 1.5 ± 0.6 log GEC across all inputs.

Significance: With similar recovery efficiencies to human norovirus GI.6 and GII.4, TCV and TV may serve as appropriate process controls for extracting and detecting noroviruses from food and environmental samples. Use of validated process controls would increase confidence in detection results and facilitate quantification of virus load, both of which are important to food safety monitoring and assessment.

P2-128 Development of a Recombinase Polymerase Amplification Assay for the Rapid Isothermal Detection of Human Norovirus

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Introduction: Reverse transcriptase qPCR (RT-qPCR) is commonly used for human norovirus (HuNoV) detection, however it is sensitive to matrix-associated inhibitors; not readily field deployable; and produces results in 1-2 hours. Recombinase polymerase amplification (RPA) uses a recombinase protein to anneal DNA for extension and amplification under isothermal conditions rapidly with potentially less sensitivity to inhibitors. Reverse transcriptase RPA (RT-RPA) has been developed for detection of other viruses but not HuNoV.

Purpose: To develop an RT-RPA assay specific to HuNoV.

Methods: Multiple primer sets corresponding to the HuNoV GII.4 New Orleans genome were identified. Using different primer combinations, the capability of amplification of purified HuNoV RNA was screened by RT-RPA (TwistDx, Cambridgeshire, UK) and a probe designed to accommodate candidate primer sets. The RT-RPA assay was used to identify the optimal primer sets that were then tested for efficiency (time to result) and limit of detection using serially diluted GII.4 New Orleans.

Results: Forty-eight primer combinations (8 candidate forward primers and 12 reverse primers) were subjected to initial screening. Eight primer pairs selectively amplified the desired product and one corresponding probe was designed. Two primer pairs (G2F5-G2R11 and G2F5-G2R12) produced significantly faster results ($P < 0.05$). At 40°C, time to results ranged from <1 minute to 7.0 minutes when amplifying purified RNA in the range of 1.5×10^{10} to 1.5×10^{6} genomic copies. When applied to dilutions of briefly heated 20% outbreak stool suspensions, signals in the range of 1.5×10^{10} to 1.5×10^{7}genome equivalent copies in 6.6 to 14.7 minutes were produced.

Significance: This is the first report of a RT-RPA assay for detection of HuNoV in relatively unpurified samples under isothermal conditions in minutes. The RT-RPA assay has potential for near "real-time" detection of HuNoV contamination in complex samples like foods and feces.

P2-129 Development and Characterization of Nucleic Acid Aptamers for the Detection of Human Norovirus across a Broad Group of Genotypes

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Introduction: Human noroviruses (HuNoV) are a leading cause of foodborne illness. There is no easy way to detect HuNoV contamination in food and environmental samples. A major reason for this is the need to concentrate and purify small numbers of viruses from the sample matrix prior to detection using reverse transcription (RT)-qPCR. Aptamers [small, single-stranded (ss)DNA or RNA molecules that naturally fold into complex three-dimensional shapes] are emerging ligands for pathogen capture. They demonstrate advantages over traditional capture ligands like antibodies, including reduced cost, ease of production and modification, and improved stability. There is also evidence that aptamers may have broader strain reactivity than antibodies.

Purpose: To create and characterize ssDNA aptamers with binding specificity to the P domain (putative capsid binding domain) of a prototype HuNoV strain.

Methods: The P protein of a HuNoV GII.4 2006b strain was used as target for selection of ssDNA aptamers. Promising candidates were characterized for binding affinity and specificity using an Enzyme-Linked Aptamer Sorbant Assay (ELASA) applied to a panel of 14 different HuNoV virus-like particles (VLPs). VLP binding was confirmed with stool samples derived from infected individuals.

Results: Two promising aptamer candidates--M1 and M6-2--containing low DG (-12.51 and -13.75, respectively) and unique secondary structures were selected for characterization. Both demonstrated strong binding affinity to GI.7, GII.2, GII.4, and GII.7 VLPs; and M6-2 showed moderate to high binding to all but one remaining VLP. Both aptamers also showed statistically significant binding ($P < 0.001$) when applied to HuNoV-positive stool samples obtained from outbreaks.

Significance: This is the first report of ssDNA aptamer selection and specificity characterization using easily-expressed HuNoV purified capsid protein. The resulting aptamers had relatively broad reactivity and performed well in clinical stool samples. They are currently being evaluated for use in capture and detection of HuNoV in foods.

P2-130 Use of Aptamer Magnetic Capture and Quantitative Real-time PCR (AMC-RT-qPCR) for Detection of Human Norovirus in a Model Food

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Introduction: Aptamers are single-stranded (ss) DNA or RNA molecules that naturally fold into complex three-dimensional shapes with binding specificity to target molecules. Interest has emerged in their use for selective concentration and purification of pathogens from complex samples. In previous work, we developed (ss) DNA aptamers with binding specificity to human noroviruses (HuNoV), the leading cause of foodborne illness. Two aptamer candidates (designated AP 25 and M6-2) showed broad reactivity in binding assays applied to a panel of genogroup I and II HuNoV strains. However, the efficacy of these ligands in capture and detection of HuNoV in foods is unknown.

Purpose: To investigate the performance of aptamers AP25 and M6-2 for detection of HuNoV in a model food system (lettuce) using Aptamer Magnetic Capture in conjunction with RT-qPCR (AMC-RT-qPCR).

Methods: Stool samples corresponding to HuNoV outbreaks (GII.4) were serially diluted and inoculated on the surface of lettuce pieces of a 9 cm^2 size. Inoculum concentration ranged from 1-5 log genome equivalent copies per sample. Viruses were pre-concentrated by sequential elution using 25 ml of 0.5 M glycine-0.14 M NaCl buffer (pH 9.0) and precipitation with 12% polyethylene glycol. After exposure to 5' biotinylated aptamer candidates AP 25 or M6-2, the virus-aptamer conjugate was captured using streptavidin coated magnetic beads. Viral RNA was extracted using the NucliSENS®easyMAG system and detected by RT-qPCR targeting the virus ORF1/ORF2 junction.

Results: The percent capture efficiency for AMC ranged from 2.5-35% for aptamer AP25 and between 1.5-7.0% for aptamer M2-6. Capture efficiency improved with decreasing virus concentration. Detection limits for the combined AMC-RT-qPCR assay were 1-2 log genome equivalent copies per lettuce sample.

Significance: Both aptamer candidates show promise for the concentration and purification of low concentrations of HuNoV from foods prior to detection using RT-qPCR.

P2-131 Patulin Determination at Screen Printed Electrodes by Square Wave Voltammetry

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Introduction: Patulin is a mycotoxin commonly encountered in juices and is not readily degraded by thermal processing. Given the stability of the toxin the main means of control is through screening raw juice or concentrate using laboratory based techniques such as HPLC. In this respect there is a demand for biosensor devices that can be used on-site for patulin detection.

Purpose: To develop and optimize a patulin electrochemical sensor based on the interaction of the mycotoxin with pyrrole

Methods: The assay was based on the formation of adducts from reacting the patulin sample with pyrrole monomer. The formation of adducts was visualized by a reduction in the oxidation peak caused by the electropolymerization of pyrrole onto the surface of a screen printed electrode. Optimization was performed in terms of pyrrole concentration, adduct formation time, pH and scan rate applied in voltammograms.

Results: The reaction of patulin with pyrrole formed an adduct that terminated polymer elongation during electropolymerization process. The observed decrease in oxidation peak correlates significantly to the concentration of patulin within the range (80ppb to 890ppb) with $r^2 = 0.92$.The detection limit is 45 ppb of patulin. The sensor response was optimal when the patulin and pyrrole were reacted for 45 mins or longer at pH 5.

Significance: The study has demonstrated proof-of-concept of an electrochemical sensor for the on-site detection of patulin. The sensor provides a further tool for inspectors and processors to minimize the introduction of patulin into the food chain.

P2-132 An Aptamer-based Dipstick Assay for the Rapid and Simple Detection of Aflatoxin B1

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Introduction: Aptamers are single-stranded oligonucleotides that can strongly and selectively bind to a target and have been regarded as a useful element in the development of biosensor and aptasensor. However, no dipstick assay based on aptamer has yet been reported.

Purpose: In this study, we developed an aptamer-based dipstick assay for the rapid and simple detection of AFB1 and validated the dipstick assay with corn samples artificially spiked with known concentration of AFB1.

Methods: A cy5-modified DNA probes and biotin-modified aptamer specific to AFB1 were designed and used to develop an aptamer-based dipstick assay for AFB1. The format of the dipstick assay was based on a competitive assay. AFB1 competes with a cy5-modified DNA probes to bind a biotin-modified aptamer specific to AFB1. The optimization of the assay was performed by testing key parameters such as the length of DNA probe, amount of the biotin-modified aptamer and cy5-modified DNA probe, working buffer, and incubation step, time and temperature. The specificity and sensitivity of the dipstick assay was tested. Sample preparation to minimize matrix effect was investigated, and corn samples spiked with AFB1 at 0, 0.1, 0.5, 1, 5, and 10 ng/g were extracted and analyzed by the assay.

Results: The aptamer-based dipstick assay for AFB1 determination was successfully developed. cy5-modified DNA with 14 mer length was selected a competitor against AFB1 to bind biotin-modified aptamer and produced sufficient fluorescence on the dipstick assay. The limits of detection for the dipstick assay were 0.1 ng/ml AFB1 in buffer and 0.5 ng/g AFB1 in a corn sample. The method was confirmed to be highly specific to AFB1, and the entire process of the assay can be completed within 30 min. Aqueous methanol (20%) provided a good extraction efficiency, and the matrix influence from corn extracts was successfully reduced through 2-fold dilution.

Significance: Recently, since aptamers have been considered as a good candidate to replace antibodies which are used in other immunoassays, the aptamer-based dipstick assay developed in this study is superior to other immunoassays with respect to its setting speed and stability. In this study, we firstly reported the development of an aptamer-based dipstick assay, and the results provide great opportunities to apply the aptamer to the development of dipstick assays.

P2-133 Development of an Experimental Method to Evaluate Inhibitory Activities of Gaseous Antimicrobial Agents

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Developing Scientist Competition

Poster

Introduction: There is a growing interest in using gaseous antimicrobial agents to inhibit the growth of hazardous microorganisms in foods. Compared to antimicrobial agents in liquid phase, antimicrobial agents in vapor phase are more effective, have fewer residues in foods, and change the sensory properties of foods less. However, there is no standard method to evaluate the lethal activities of gaseous antimicrobial agents.

Purpose: This study was done to develop a method to determine the minimal inhibitory concentrations (MIC) and minimal lethal concentrations (MLC) of gaseous antimicrobial agents.

Methods: Experimental apparatus with upper and lower polycarbonate chambers was constructed. Nutrient agar containing 0.025% bromocresol purple and 1% glucose was poured into wells in the upper chamber and *Escherichia coli* O157:H7 (5 log CFU/well) was inoculated. Sterile filter discs were placed on the wells in the lower chamber and essential oils (EO) serially diluted were deposited. The chambers were sealed and incubated at 30°C for 48h. After incubation, the lowest concentration of EO which did not change the color of agar into purple was determined. The agar whose color was not changed was streaked on nutrient agar to determine the MLC.

Results: MIC values of 30 essential oils in vapor phase against *E. coli* O157:H7 were determined. Vapor of cinnamon bark oil showed the lowest MIC (0.0391 µl/ml). The order of MICs of EOs in vapor phase was cinnamon bark < thyme-thymol < peppermint = oregano < thyme-linalool = clove = spearmint < tea tree = thyme Spanish = clary sage. The order of MLCs was cinnamon bark < thyme-thymol < oregano < peppermint = thyme-linalool < clove = spearmint = tea tree = thyme Spanish < clary sage.

Significance: An experimental apparatus and standardized method to determine MIC and MLC of gaseous antimicrobial agents were developed.

P2-134 Development of a New Device for the Rapid Detection of Heterofermentative and Homofermentative Lactic Acid Bacteria

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Introduction: A new Soleris® vial was developed to rapidly detect heterofermentative and homofermentative lactic acid bacteria contamination in dressings, condiments, and deli and packaged meats. 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 lactic acid-producing microorganisms can be detected within 72 hours using the Soleris instrument system.

Purpose: The purpose of this study was to develop and assess the performance of a device for the rapid detection of lactic acid-producing bacteria in the dressing, condiment, and deli and packaged meat industries.

Methods: Experiments were performed to select the optimal growth medium and indicator chemistries for the most rapid detection of lactic acid bacteria. Inclusivity studies used 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/exclusivity test panel of ATCC organisms included *Lactobacillus*, *Leuconostoc*, *Pediococcus*, yeast, and mold. The Soleris instrument detection times were as low as 16.2 hours for an inoculum level of 55 CFU/mL for *Lactobacillus fermentum* ATCC 9338, and as high as 77.6 hours for 85 CFU/ml of *Lactobacillus fructivorans* ATCC 8288. Naturally contaminated deli meats (10^2 to 10^4CFU/g) produced a range of detection times between 9.7 and 16.8 hours. The food matrices tested did not interfere with the vial test.

Significance: The new Soleris Direct Lactic Acid vial provides a system for the rapid detection of lactic-acid producing microorganisms in <50 hours, with the exception of *Lactobacillus fructivorans* ATCC 8288, compared to the standard incubation time of 5 days for agar plates.

P2-135 Rapid Quantitative Enumeration of Aerobic Count Bacteria in Foods

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Introduction: Aerobic count bacteria are ubiquitously present in a variety of food matrices and serve as indicators for food spoilage. These microorganisms can grow in a wide range of temperatures and pH values thus having the potential for substantial economic losses to the food industry. These organisms have become important indicators for monitoring food quality but the 48 hour incubation required by traditional enumeration methods poses a burden on food producers. A novel dehydrated film medium was developed to address the need for rapid (24 hour) detection of aerobic bacteria. The detection technology was optimized to overcome the inherent limitations associated with over expression of extracellular enzymes by certain spore formers that results in liquefaction of the dehydrated film media.

Purpose: This study was performed to comparatively enumerate aerobic count bacteria using a new dehydrated film medium and reference methodology as described in FDA/BAM and ISO 4833.

Methods: The method comparison was conducted using a variety of naturally contaminated food matrices (n > 75) with the new dehydrated film test method and a reference method (FDA/BAM and ISO4833). Each food sample was serially diluted in Butterfields buffer. One milliliter (ml) each of the sample or its dilution was used for the candidate and the reference method (Standard methods agar; pour plate method).

Results: Results were statistically comparable between the new dehydrated film method at 24 hrs to Standard Methods Agar at 48 hours (at 32°C or 35°C) or 72 hours (at 30°C) with no significant differences as indicated by the *P* values (> 0.05) and regression analysis.

Significance: Rapid quantitative detection of aerobic count bacteria using the new dehydrated film method was evaluated using a large number (n > 75) of naturally contaminated foods. The new method was found to provide actionable results in a shorter period of time, with significantly improved interpretation, without sacrificing performance which is critical for food processors.

P2-136 A New Rapid Quantitative Method For Enumeration of Aerobic Bacteria in Aseptically Packaged Purees/Juices

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Introduction: There is a continuing search for rapid methods to detect contamination in aseptically packaged products such as juices/purees. To verify the effectiveness of the thermal process it is essential to test finished product to determine if potential contamination has survived the process. This involves holding the product until bacterial testing has been completed. This usually takes a minimum 48h, costing money for storage and warehouse fees.

Purpose: This study was conducted to evaluate a new oxygen-sensing method for the quantitative detection of aerobic bacteria that might be present in aseptically packaged products within 12 hours.

Methods: A cocktail containing 0.5 ml of *Escherichia coli* and *Enterobacter faecium* was used to inoculate sterile 12 ounce aseptic juice/puree packages. Three levels of inoculum were used (10^1, 10^2 and 10^3dilution) and done in duplicate. Nine mls of Buffered Peptone Water and 1 ml of

each dilution were placed into a 15 ml sensor vial. The vials were placed into the system. Concurrent plate counts were performed for quantitative comparison.

Results: Three separate trials were run with correlation coefficients of plate counts to time-of-results greater than 0.80 with a maximum total test time of 12 hours.

Significance: This method has been proved to produce quantitative results for the enumeration of bacterial contamination in aseptically processed juice/purees in hours rather than days, allowing processors to release product quicker therefore, saving time and money.

P2-137 New Technology for Rapid Detection/Identification of Bacteria and Yeast in Food, Beverage and Water

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Introduction: Methods for a rapid, sensitive and reliable detection and quantification of microorganisms and pathogens in food, beverages and water are receiving increasing attention. The sandwich hybridization method used in the HybriScan Test system is a suitable alternative for such analysis. This test method is independent of the influence of sample matrices, and is able to distinguish between live and dead cells. Furthermore, the detection of non-culturable microbes is possible.

Purpose: A rapid test for the detection of foodborne pathogens like *Salmonella, Campylobacter, Listeria* and *Cronobacter* spp., and counting of *Legionella* in water, including the most relevant species, *L. pneumophila* was needed. The test is needed for early detection of pathogens, and for better and economical quality control.

Methods: The method is based on the detection of rRNA via hybridization events and specific capture and detection probes. Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labeled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin-labeled detection probe provides an enzyme-linked optical signal read out. Detection results from application of anti-DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB (3,3',5,5'-tetramethylbenzidine). Photometric data are measured at 450 nm and compared with standard solutions. The HybriScan software enables easy measurement and data analysis.

Results: Food samples were analyzed with the method and compared to the culture based method according to 64-LFGB. Five different food categories were tested. 355 food samples were analyzed and compared to culture-based method according to 64-LFGB. Validation was according to ISO 16140:2003 (ASU L00.00-22). Results of validation showed a relative accuracy of 99.2 %, relative specificity of 98.5% and relative sensitivity of 99.6%.

Significance: The new method is an economical, high throughput, 96-well microplate format system. The test is performed in less than 3 hours (in addition to the prep time) and offers significant time saving compared to cultivation-based assays.

P2-138 Concentration of Bacterial Pathogens Using Apolipoprotein H

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Introduction: Concentration of bacteria from food or environmental samples prior to detection could reduce or even eliminate the need for cultural enrichment. A broadly reactive ligand with the ability to concentrate a variety of microbes from relevant sample matrices could facilitate this type of sample preparation. The human plasma protein Apolipoprotein H (ApoH) has been shown to have a high affinity for a number of Gram- and Gram+ bacteria.

Purpose: To investigate the utility of ApoH conjugated to magnetic beads for the capture and concentration of select foodborne bacterial pathogens.

Methods: Overnight cultures of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* serovar Enteritidis, and *Staphylococcus aureus* were serially diluted in proprietary binding buffer to concentrations of 10^3, 10^5 and 10^7CFU/100 µl. Suspensions were supplemented with 10µl of ApoH conjugated magnetic beads (ApoH Technologies, Villeneuve St Georges, France) and incubated for 60 min at 4°C with rotation. The beads were captured by magnet and washed twice. Both bead and supernatants suspensions were retained for cultural enumeration of bacteria. An aliquot of the beads was also subjected to DNA extraction followed by detection of each pathogen using a SYBR green qPCR method targeting the 16S rDNA gene.

Results: Based on loss to supernatant, the ApoH beads showed high capture efficiency (73.4-100%) for all four pathogens tested and at all three concentrations (10^3, 10^5 and 10^7 CFU/100 µl.) In most cases, there were no statistically significant differences in capture efficiencies when comparing pathogens or initial cell concentration ($P > 0.05$, n = 3). The SYBR green qPCR results were more variable but in general, assay detection limits after ApoH capture and qPCR were approximately one log CFU higher compared to input cell numbers.

Significance: ApoH conjugated magnetic beads show promise for concentration of bacterial pathogens in preparation for detection using cultural methods or qPCR.

P2-139 Hyperspectral Microscope Imaging Methods for Rapid Identification of Pathogenic Bacteria

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Developing Scientist Competition

Introduction: Hyperspectral microscopy has the potential to identify pathogenic bacteria on a cellular level, based on the differences in each species' unique spectral signature obtained from microcolony samples. Compared to current identification protocols the rapid nature of this methodology could potentially reduce the standard bacterial identification time.

Purpose: This research was designed to evaluate the spectral differences in five pathogenic bacteria species, and determine through image processing and multivariate data analysis if the samples can be differentiated.

Methods: Five species of bacteria (*S.* Enteritidis, *E. coli, P. putida, L. innocua.,* and *S. hyicus*) were analyzed through a hyperspectral microscope equipped with a dark-field illuminated halide lighting source and acousto-optical tunable filter. Bacteria colonies were picked from plate count agar and suspended in autoclaved water, followed by air drying 10 µl of the suspension on a microscope glass slide taking approximately fifteen minutes. A total of 89 contiguous images were collected every 4 nm between 450-800nm, followed by multivariate data analysis and analysis of variance (ANOVA).

Results: Principle component analysis was conducted on 125 cellular data sets with clear cluster separation noticeable, and Mahalanobis distances between sample clusters ranging from 10.971 to 85.186. Partial least squares regression was used to validate a training set of data (n = 125) with root-mean squared error of validation 0.3319 and a R^2 value of 0.9955. One-way ANOVA was performed on a 2-band (546 and 590 nm) relative ratio of the spectra with all samples significantly different ($P < 0.05$), except for comparing the two gram-positive species *L. innocua* to *P. putida*.

Significance: This study shows that bacterial species possesses unique physiological characteristics that affect light scattering, and can therefore be classified rapidly through hyperspectral microscopic image analysis followed with chemometric data processing.

P2-140 Microfluidic Chip-based Optical Biosensor for the Multiplex Detection of Foodborne Pathogens

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Introduction **:** Foodborne pathogens are responsible for the majority of foodborne illnesses and deaths and seriously threaten public health worldwide. For effective prevention of foodborne infections, new alternative approaches that overcome drawbacks of conventional detection methods are demanded to develop simple, sensitive, time-saving, and cost-effective methods.

Purpose **:**With the aim of developing advanced method, we have devised a new sensing system that simultaneously detects 12 foodborne pathogens by using optical signals generated in designated wells for each pathogen on microfluidic chip.

Methods **:**Each capture probe specific for the genomic regions of 12 pathogens was spotted on the wells (400 μm in diameter per well) of micropatterned-COC (cyclic olefin copolymer) chip and directly immobilized by UV irradiation. The biotin-labeled target DNAs for the pathogens were prepared by multiplex PCR using biotin-labeled primers, then the target DNAs flowed on the chip and were incubated for 5 min at 60°C. Streptavidin and gold nanoparticles conjugated with biotin then sequentially flowed and were respectively incubated for 20 min at room temperature. Next silver solution flowed and silver aggregation only on the surface of gold nanoparticles leads to significant reduction of light transmission into the chip and high contrast signal visible under optical scanner.

Results **:** By employing the method, all 12 pathogens were correctly identified through the optical signals at the correct positions of the wells without any interference caused by non-specific binding or noise, demonstrating its high selectivity. Furthermore, pathogen concentrations were conveniently determined using optical intensities induced by silver aggregation proportional to the amount of target pathogens.

Significance **:** The results demonstrated that the new method displays high sensitivity (limit of detection= 5 x10^2 cells), precision (<10%), and convenience by automated multiplex analysis within 90 min. By eliminating the requirement of labor-intensive and time-consuming procedures, the new approach should be widely applicable in pathogens sensing.

P2-141 Fabrication and Potential Application of Biomimetic Surface for Probing Plant-Bacteria Interactions

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Developing Scientist Competition

Introduction: Understanding of plant-bacterial interactions is of critical importance for developing effective control measures against infectious diseases caused by foodborne human pathogens. However, limitations of existing scientific tools to access and evaluate natural plant tissues, and the large variations in hierarchical microstructure and topographical features among plant leaf surfaces significantly hinders the understanding of plant-bacteria interfacial interactions and development of effective intervention technologies.

Purpose: Develop novel biomimetic material that could replicate surface chemical and structural information from natural plant tissue to facilitate research studies on bacteria-substratum interactions and development of effective pathogen inactivation technologies.

Methods: In this study, we developed a two-step replica molding method for rapid fabrication of polydimethylsiloxane (PDMS) and agarose (AGAR)-based biomimetic surfaces, using spinach leaf as a model. The potential application of those biomimetic surfaces for food safety research was further evaluated.

Results: Both polymers successfully mimicked the leaf surface microstructure, while each possesses unique chemical, physical, and biological features. PDMS biomimetic surfaces provide structural durability for scanning electron microscopy examination, comparable surface wettability for coating development, and real-time monitoring capability by incorporation into a micro-fluidic device. AGAR biomimetic surfaces are suitable for bacterial growth, recovery, and quantification studies. AGAR biomimetic surfaces demonstrate great capacity for investigating the effect of surface topography on the survival and inactivation of *Escherichia coli* cells during biocide treatment.

Significance: Overall, this technology facilitates reproducibility of experiments involving disinfection and attachment/release of microbes from plant surfaces, but without any of the leaf-to-leaf or plant-to-plant variability that confounds experiments with real produce.

P2-142 Optimizing Sample Plans to Improve Microbiological Safety in a Food Processing Plant

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Introduction: *Salmonella* and *Cronobacter sakazakii* are two leading causes of foodborne illness associated with low-moisture foods, including infant formula. Both causative organisms can persist in food manufacturing environments and contaminate finished product if programs are not in place to limit their introduction and control their spread. Microbial sampling and testing of the process environment and finished product are valuable for identifying and preventing contamination.

Purpose: An environmental sampling and monitoring program is an important tool that food manufacturers use to determine the effectiveness of their sanitation practices and pathogen control efforts. The objective of this research was to develop guidance for initiating and evaluating an environmental sampling plan for an infant formula process.

Methods: An interactive spreadsheet tool for designing sampling monitoring plans for Salmonella (qualitative) and Enterobacteriaceae (quantitative) was developed using Microsoft Excel and Visual Basic for Applications (VBA). Microbiological environmental sampling plans (9) were based on the answers to a series of questions related to product hazards, processing risks and controls, and knowledge of appropriate microbiological sampling and testing protocols. Furthermore, these initial sampling plans were related to the volume of product and size of the processing facility. Guidelines were designed to support the instructions for selecting and modifying a sampling plan.

Results: Additionally, the tool could be used to record qualitative and quantitative sample test results, and to alert the user to adjust the sampling plan, if necessary, based on monthly test summaries. The sampling tool provides a simple method for selecting an appropriate environmental

sampling plan (samples per zone per month) based on product and process risk level and production volume, and provides a rationale and guidance for modifying these plans.

Significance: Effective, well-designed sampling plans and trend analysis of sample test results support food processors' decisions for implementing controls to enhance food safety.

P2-143 Preparation and Advantages in the Use of a Dry Inoculum for Challenge Testing

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Introduction: At times it is not practical to use a wet inoculum due to time constraints in growing and preparing the inoculum as well as variability culture levels. The freeze drying protocol was developed to provide a dry stable bacterial inoculum on an inert material (talc) for use on dry products and/or materials that cannot be mixed with water.

Purpose: The objectives of this protocol were to evaluate the stability of a dry inoculum of pathogens such as *Salmonella* spp. and provide a means to prepare consistent cultures for inoculating dry products where it was important to not alter the physical conditions of the material.

Methods: Talc has demonstrated superior characteristics as a carrier due to its fine uniform particle structure. The culture is grown on agar plates and the growth is harvested during the stationary phase via washing the surface of the agar plates. The collected organisms are combined with a small amount (<1.0 grams) of the dry carrier (talc) and frozen at -76°F/-60°C. After freezing the cultures are freeze dried, pulverized with a mortar and pestle and brought to desired volume in talc. The inoculum is then counted and held for use under vacuum in a desiccator at 39°F/4°C.

Results: Results indicated that this freeze dried bacterial culture is stable for an extended period of time, greater than 3 months at 39°F/4°C.

Significance: The use of a dry culture can mimic the mode of contamination of food in dry manufacturing and storage. A dry inoculum can be used with materials that are not compatible with water (triacetin, chocolate, medium chain triglycerides, dried fruit, etc.).

P2-144 Microbial Profiling of Raw Tomatoes and Tomato Products by Next Generation Sequence

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Introduction: Aerobic Plate Count (APC) is the traditional method used to estimate the microbial load of an agricultural commodity. Culture independent molecular technologies, such as Next Generation Sequence (NGS) based 16S rRNA sequences or metagenomics, have revolutionized our ability to answer the question of "who is there" in microbial communities, and provides extra information on population structures and microbial composition of a particular food product.

Purpose: The purpose of this study was to evaluate NGS based 16S rRNA sequences as a tool to assess microbial flora of raw tomatoes and tomato products.

Methods: Raw tomatoes were sampled from three different lots over a two-month period and tested for APC by pour plating methods. Three different lots of raw processed products, including diced tomatoes, diced tomatoes with chilies, and tomato paste, were also collected and tested for APC. All samples collected were subjected to total DNA extraction, PCR amplification using universal 16S rRNA primers, and sequenced on NGS platform using metagenomics workflow. Taxonomy at genus level was assigned for each sample against the Greengenes Database.

Results: The average APC of raw tomatoes for three lots was 7.33 ± 0.52 logs CFU/gram. The average APC for diced tomatoes, diced tomatoes with chilies, and tomato paste was 6.01 ± 0.63, 6.89 ± 0.60, and 4.89 ± 0.92 logs CFU/gram, respectively. The 16S rRNA sequences showed consistency of microbial flora for the same type of products among three different lots tested. These results also show that raw tomatoes had the most microbial diversity and many microbial populations were reduced or eliminated after the process of tomatoes into paste (before the heating step) even though only a 2 log APC reduction was observed. However, not many microbial population changes were observed among raw tomatoes, diced tomatoes, and diced tomatoes with chilies (before the heating step). The alterations in the microbial populations are likely due to differences in processing between the paste and diced products.

Significance: These data suggest NGS based 16S rRNA sequencing may be a useful tool for the assessment of microbial diversity of food products and these data may eventually be useful to evaluate processing parameters specific to different product types and desired quality attributes.

P2-145 HiDtect™ Rapid Identification Discs for Confirmation of Food Pathogens

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Introduction: Rapid Identification of food borne pathogens especially *Escherichia coli,* have been considered to be a major challenge in food industries. Classical method of bacterial identification involves the use of enriched and selective medias for the growth of organisms and subsequent confirmation using series of biochemical tests, that would take 48-72 hrs for the completion. Present study describes the use of HiDtect™, a novel chromogen based paper disc technology for the rapid, qualitative detection of *E. coli*.

Purpose: For rapid detection and confirmation of *E.coli* in water and food samples.

Methods: Three sets of chromogenic discs such as DT 006, DT007 and DT008 were prepared using chromognic, fluorogenic and dual technologies. *ATCC 25922* and six other isolates of *E.coli* were used for the inclusivity study whereas *Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) and Salmonella Typhimurium (ATCC 14028)* were used for Exclusivity study. All the cultures were grown on Plate Count Agar. Fully grown cultures were replicated on the three different chromogenic discs by placing the discs over the colonies for 30 sec to 1 min. These discs after replication were incubated at 35-37°C for 1-4 hours and observed for colour change (DT006), fluorescence (DT007) and both (DT008).

Results: The incubated discs were checked for the presence of *E. coli* by observing the blue colouration (in DT006), fluorescence (in DT007) and both blue colouration and fluorescence (in DT008) of the replicated colonies. All the tests were performed at least in triplicates and presented as qualitative observations. Results (Table 1) obtained shows that these discs have high specificity and reproducibility in comparison with the routine biochemical methods.

Table 1: Results of various discs after replication of the colonies

Organism	DT006 Colourchange	DT007 fluorescence under UV light	DT008 Colourchange/ fluorescence under UV light
E. coli (ATCC 25922) and six isolates	blue	Positive	Positive
Pseudomonas aeruginosa (ATCC 27853)	colourless	Negative	colourless / Negative
Staphylococcus aureus (ATCC 25923)	colourless	Negative	colourless / Negative
Salmonella Typhimurium (ATCC 14028)	colourless	Negative	colourless / Negative

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Significance: Chromogenic discs have advantages of saving costs, time and labour over the conventional methods for identification of *E.coli*.
- Direct application without any preparations.
- Confirmed results are obtained within 1-4 hours in comparison to 48-72 hrs required by traditional methods.
- The technology is highly sensitive, that can identify even a single developed colony.
- Discs after identification can be stored for further traceability without any deterioration in colour.

P2-146 Comparison of ELISA Technologies for Detection of *C. botulinum* Toxins A, B, E, and F in Foods

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Introduction: *Clostridium botulinum* toxin (BoNT) is a powerful neurotoxin that causes severe foodborne illness, including botulism, in humans. Seven types of neurotoxins have been identified in *Clostridium botulinum*, but only types A, B, E, and F are known to cause disease in humans. Effective commercially available validated kits for these BoNT types are necessary in the event of a large scale food emergency involving BoNT.

Purpose: This study was a parallel comparison between the DIG-ELISA *C. botulinum*toxin kits and the commercially available Tetracore ELISA A, B, E, and F kits for detection of BoNT in foods.

Methods: Six brands each of canned chili, sausage, and luncheon meat were fortified at five levels of BoNT A, B, E, and F, and cold stressed overnight. Each sample was replicated eight times over four days. Samples were homogenized with GBS and toxins were extracted via centrifugation. The aqueous layers were filtered, prepared, and analyzed in parallel according to manufacturer's instructions on the Tetracore and DIG-ELISA methods for each toxin specific type.

Results: Limit of Detection (ng/g) of BoNT Types A, B, E, and F in Foods

ELISA	Chili				Luncheon Meat				Sausage			
Method	A	B	E	F	A	B	E	F	A	B	E	F
Tetracore	25	5-10	5	5	>50	50	500	5	>50	50	100	5
DIG	10	>50	5	5	10-25	>50	5	5	10-50	>50	5	5

Significance: While the DIG-ELISA method was more sensitive in the detection of toxin type A in all matrices, the detection limits of toxin types E and F were comparable in both methods. The Tetracore ELISA method exhibited greater sensitivity in the detection of toxin type B in all matrices evaluated.

P2-147 Withdrawn

P2-148 Optimization of BAX® System Enrichment Media for *Listeria monocytogenes* and Genus *Listeria* Assays

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Introduction: Food legislation in many countries rigidly enforces limits for *Listeria monocytogenes* and other species in genus *Listeria*. The DuPont™ BAX® System includes a range of PCR assays for detecting genus *Listeria* and *L. monocytogenes* in food and environmental samples. As part of its commitment to customers, DuPont strives to continually improve its products to satisfy the evolving needs of the industry and exceed customer expectations.

Purpose: The purpose of this study was to reduce the enrichment times used with the system's method for detecting genus *Listeria* and *L. monocytogenes*. Variations of standard enrichment media and their selected components were examined for optimal outcome.

Methods: A variety of standard enrichment media were compared to the approved proprietary media for recovering heat stressed *Listeria* cells by pure culture growth and plating. The top performing standard media was then evaluated to determine if the addition or alteration of media components could further improve performance with our PCR Assay for *L. monocytogenes*24E. Components evaluated included pH buffering reagent, nutrients, energy and carbon source, salts, antimicrobials and other supplements.

Results: The optimized formulation returns more true positive calls than the certified proprietary media by the system's PCR Assay for *L. monocytogenes* 24E on ham ($P = 0.001$ by z-test), smoked salmon ($P = 0.028$ by z-test), raw shrimp ($P < 0.001$ by z-test), hotdog ($P < 0.001$ by z-test) and cheese ($P < 0.001$ by z-test) at 22 hours post-enrichment that has not been validated for the certified proprietary media.

Significance: These findings suggest it is possible to further reduce the enrichment times of the system for detecting *Listeria* and *L. monocytogenes*, while continuing to offer the same accuracy, reliability and ease of use as the original protocols.

P2-149 Screen-printed Electrode-based Aptasensor for Rapid Detection of *Escherichia coli* O157:H7 in Foods

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Developing Scientist Competition

Introduction: *Escherichia coli* O157:H7, as one of the life-threatening foodborne pathogens, has continued to be a serious food safety issue worldwide. A rapid and sensitive method for on-site detection of this pathogen is demanded.

Purpose: Therefore, an impedance aptasensor based on the use of magnetic nanobeads for separation and screen-printed interdigitated electrode for measurement was developed for the rapid detection of *E. coli* O157:H7 in food products.

Methods: Streptavidin coated magnetic nano-beads (MNBs) (150 nm) were functionalized with biotinylated *E. coli*-aptamers, and then were mixed with samples containing *E. coli* O157:H7. A magnetic separator was applied to isolate and concentrate the captured target bacteria. Concanavalin A-glucose oxidase (Con A-GOx) complex was employed as an amplifier to form *E. coli*-Con A-GOx sandwich through Con A-glycan interaction. The yielded sandwich complex was then transferred to a glucose solution to trigger an enzymatic reaction to produce gluconic acid, which ionized to increase the ion strength of the solution, thus decreasing the impedance on a screen-printed interdigitated electrode. By measuring the impedance change (DZ) over a 30-min reaction, the correlation of DZ with the concentration of *E. coli* was determined.

Results: Our results showed that the developed aptasensor was capable of specifically detecting *E. coli* O157:H7 within 1.5h, and can detect as low as 200 microbial cells in a 200 µl sample (10^3 CFU/ml). Ongoing researches focus on the target bacteria in different foods and a prototype of the instrument.

Significance: This provides a practical method for rapid, sensitive, and on-site detection of *E. coli* O157:H7.

P2-150 Content Analysis of Food Safety Education Materials

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Introduction: Noroviruses (NoV) cause over five million cases of foodborne disease each year. Education is one method for decreasing the burden of illness due to NoV infection. Thousands of education materials that focus on strategies to prevent foodborne disease are available, however, it is unknown if these materials correctly address prevention and control strategies for NoV.

Purpose: The purpose of this study was to identify if and how prevention and control strategies for NoV were addressed in education materials targeting adult consumers.

Methods: All food safety education materials targeting adult consumers listed in the USDA Education and Training Materials Database as of Summer 2013 were included in our sample (N = 155). Each artifact was coded by two trained staff members using a coding sheet created specifically for this project. Inter-rater reliability was calculated as percent agreement between coders (94.5%). Frequencies were tabulated using SAS version 9.3.

Results: Most (80.6%) artifacts did not mention the word "virus" or a word containing "virus" and NoV were only mentioned in 3.53% of the artifacts. Most did mention handwashing (66.7%) but most did not mention the length of handwashing (63.1%) or minimizing contact with persons when they are sick (96.8%). The majority of artifacts did not address sanitizing of food-contact surfaces (73.9%), and most did not suggest a method for cleaning up vomit or fecal matter (90.1%).

Significance: If the number of cases of foodborne disease is to decrease, existing education materials must be revised to include prevention and control strategies for NoV, the number one cause of foodborne disease in the U.S.

P2-151 Development of an Online Food Safety Training for School Gardens

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Introduction: School gardens are increasing at a significant rate around the country. Most of the students that will be working in these gardens have little to no experience with produce or food safety training. Implementation of training is necessary due to the high food safety risk associated with produce items with an at risk population.

Purpose: The objective of this study was to develop a one-hour on farm food safety online module, which would inform k-12 students on various hazards associated with production and harvesting of fresh produce.

Methods: A one-hour on farm food safety online module for school age kids was developed using GAP approved educational and governmental resources. Quizzes for students and a user manual were created for facilitators and instructors on how to effectively perform the food safety training. Upon completion of the module and manual, a pilot study was performed at two K-12 school districts in Iowa along with a six member expert panel on education, agriculture, nutrition and food safety steering committee to test effectiveness of the preliminary impacts of the online training and user manual. Participants were asked to complete an online survey consisting of scale likelihood questions and open-ended questions for the facilitators and instructors to determine the effectiveness of the online module and the supporting materials. Statistical analysis was performed on the results.

Results: Survey results indicate that the online module will significantly increase the knowledge of students related to hazard associated with produce food safety ($P < 0.05$). The topics, graphics, design, length, and webpage were appropriate for the age group. Additionally, the resource guide and quizzes will be helpful and increase the knowledge and confidence of the facilitators of the training ($P < 0.05$). The group indicated that specific graphic and content material changes should be made within the module.

Significance: This online module is a first step toward educating the youth about food safety in the garden. Knowledge on hazard prevention will reduce the risk of produce contamination and decrease produce outbreaks from occurring in school gardens.

P2-152 Farmers Market Food Safety: Educating and Engaging

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Developing Scientist Competition

Introduction: There is a strong movement towards purchasing fruits and vegetables directly from the producer in venues such as farmers' markets; however, fresh produce is one of leading causes of foodborne illness. FSMA guidelines exempt small farmers from adhering to produce safety standards but small farms are still responsible for identifying potential hazards associated with the produce and implementing preventive controls. This has been a source of confusion and frustration for many small farmers.

Purpose: The overall goal of this project is to develop best food safety practices educational material for small farmers' and market vendors. Animated educational videos were designed to stimulate the learning capacities of both auditory and visual learners. Based on the videos, info-sheets were designed for posting at crucial locations within farmers markets.

Methods: A survey was designed to identify good and high-risk consumer and vendor practices at farmers markets. This survey was carried out in a passive manner by 4 different investigators at 7 farmers markets in Texas. Based on these results, 20 food safety scripts were composed for the educational videos and info-sheets. The videos were designed using animation software to actively engage and educate the audience simultaneously. The info-sheets were designed based on the animations.

Results: The results demonstrated high-risk practices such as poor personal hygiene and animal exposure to produce. Positive initiatives such as presence of hand washing stations, hand sanitizers, and gloves available for the consumers and vendors were also observed. The videos and info-sheets highlighted "good ideas" or positive behaviors and "bad ideas" or high-risk consumer and farmer behaviors.

Significance: By following positive food safety practices outlined in the videos and info-sheets, small farmers' can reduce the risk of consumers contracting foodborne illness. Small farmers can generate a strong and loyal customer base by providing local, healthy, and safe food.

P2-153 Food Safety Guidelines for Farmers Markets in the United States: A Need for Standardization

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Introduction: Consumer support for the local food movement and farmers markets has grown exponentially in the past decade. Although there have been few foodborne disease outbreaks (FBDO) directly linked to farmers markets, the majority of FBDOs are never identified. Therefore, farmers market vendors (i.e., farmers and prepared food workers) and managers need to ensure that appropriate good handling practices and best management practices are followed to protect public health from the contamination of fresh produce commodities sold at the market.

Purpose: The overall goal of this pilot study was to determine whether farmers market vendor guidelines include recommendations for good food safety practices specific to the handling of fresh produce.

Methods: Farmers markets (n =12) across the United States were randomly selected and vendor guidelines were analyzed for food safety related information/standards and procedures. The contents were recorded and a comparative analysis was conducted.

Results: Overall, only 8.3% of farmers markets state explicit food handling procedures that would enhance food safety. Only 33.3% of farmers markets have information for vendors regarding inspection of farms and products for verifying compliance with federal, local and national policies. Last, the majority of the markets (91.7%) do not restrict vendors from selling fresh poultry and meat products that can be leading causes of viral and bacterial cross contamination on to fresh produce.

Significance: These results demonstrate a need for basic standardization of food safety instructions, guidelines and regulations at farmers markets. It is crucial for farmers market managers to be clear and explicit in their communication with vendors regarding good food safety practices and strive to protect public health. These data contribute to our digital compilation of rules and guidelines for vendors for each state as part of USDA NIFA 2013-68003-21288.

P2-154 The Influence of Television Celebrity Chefs on Consumers' Food Safety Practices in the Home

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Developing Scientist Competition

Introduction: Television cooking shows have become increasingly popular. Previous research in Canada and the United States documented that chefs frequently exhibit poor food handling behaviors. Consumers watch cooking shows for entertainment as well as education, and therefore may have an increased chance of adopting an unsafe practice and thereby increasing the risk of foodborne illness.

Purpose: This study investigates the food handling errors of four well-known celebrity chefs, and consumer's attitudes toward these errors.

Methods: A content analysis of 60 television cooking shows was performed. A scale, based on The Partnership for Food Safety Education's Four Core Practices: Cook, Clean, Chill, Separate was developed, validated, and used to evaluate the cooking shows. Focus groups were used to obtain qualitative data about consumer attitudes towards the chefs' behaviors based upon viewing clips from each chef.

Results: The content analysis indicated that some chefs continue to practice dangerous food handling behaviors. Lack of hand washing prior to food preparation or after handling raw meat, failure to use a cooking thermometer to confirm doneness of meat, and cross contamination are frequently observed. Instances of cross-contamination often included handling of utensils or other items, such as salt or cooking oil after handling raw meat. Focus groups confirm that some consumers are unaware of the breach of safety protocol and admit they might follow similar practices while others report thinking less of the chef's expertise when mishandling occurs.

Significance: Consumers view celebrity chefs as role models, utilize information transmitted during cooking shows, and often practice similar behavior in their own kitchens. Chefs' poor food handling practices could increase the risk of foodborne illness from food prepared at home.

P2-155 Impact of Food Safety Messages on Consumer Food-handling Behaviors

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Developing Scientist Competition

Introduction: Foodborne illnesses affect 48 million U.S. citizens annually. Improper food handling and hygiene practices during consumer meal preparation lead to contamination of the kitchen environment and can result in foodborne illness. Observational research indicates that some consumers may be knowledgeable about food safety, but safe food handling recommendations are not widely followed.

Purpose: This study was conducted to determine the impact of four key messages (clean, separate, cook and chill) on consumers' food handling behaviors and to measure the spread of microbial contamination from raw meats to both ready-to-eat (RTE) foods and the kitchen environment during a typical home meal preparation.

Methods: Participants (n = 123) were either exposed to the standard food safety messages through a formal presentation, video clips, or were not exposed (control). Participants subsequently prepared a main dish from raw chicken or ground beef accompanied by a RTE fruit salad. Activities were video recorded and behaviors scored. Raw meat ingredients were previously inoculated with a non-pathogenic *Lactobacillus casei* culture to track cross-contamination.

Results: Approximately 90% of the fruit salads prepared by all participants became contaminated. Hands and kitchen towels were identified as major contamination sources in the kitchen. There was a significant difference ($P = 0.03$) in the least square mean hand washing scores between the group exposed to the four messages via formal presentation and the control group. More risky food handling behaviors were seen among people handling chicken than those handling beef based on differences ($P \leq 0.01$) in the least square mean cross-contamination scores of participants preparing entrees containing chicken (M = 0.87) compared to beef (M = 1.02).

Significance: Methods of relaying food safety guidance to consumers differ in their impact on consumer behaviors. Observational studies utilizing methods that quantify food safety risks must be employed to improve effective messaging.

P2-156 Investigating Positive Deviance Intervention to Change Consumer Food Safety Awareness and Behavior

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Poster

Introduction: The positive deviance focus group is a novel food safety educational intervention that allows participants to discuss their food handling behaviors, and decide to try recommended practices modeled by people like themselves.

Purpose: The objective of the study is to evaluate the effectiveness of an educational intervention utilizing positive deviance to increase consumer safe handling behavior.

Methods: A pilot group of consumers participated in three 1-hour focus group sessions, completed pre/post surveys, and performed two food safety take-home tasks. The survey assessed knowledge, attitude and personal hygiene before and after the intervention. The take-home tasks addressed their behavior and attitudes toward following food safety recommendations regarding temperature control and cross contamination.

Results: Pre/post survey results indicated that consumer's food safety knowledge and awareness increased after the intervention. The first take-home task was to measure the temperature of different spots of their home refrigerators using a refrigerator thermometer, and measure the temperature of home cooked items using a cooking thermometer. Prior to using the cooking thermometer participants stated "I don't think I need it." After completing the take-home task, consumers reported "it is fun to see what temperature my food is now" and "I will definitely use it in the future." The second take-home task was to use separate cutting boards for meat and produce. Participants found different ways to separate the cutting boards; instead of separating by meat vs. produce, some participants preferred to separate by 'prepared to cook' vs. 'ready to eat.'

Significance: Based on the qualitative data, this positive deviance intervention increased consumer's knowledge and safe handing behavior. Plans are to expand this approach to a larger audience of high risk individuals.

P2-157 Need for Education about Noroviruses: Findings from a Nationally Representative Survey of U.S. Adults

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Introduction: Noroviruses (NoV) are the leading cause of foodborne disease in the United States, sickening over five million people each year. Consumer education is one method for decreasing cases of illness. Establishing a baseline of consumers' NoV knowledge is essential to informing the development of effective education materials.

Purpose: The purpose of this study was to determine the relationship between select demographic characteristics and perceived susceptibility and severity of NoV infection.

Methods: A nationally-representative, Web-enabled panel survey of U.S. adults (N=1,051) was conducted to collect information on consumer demographics and personal beliefs regarding contracting a NoV infection. As part of a larger 27-item instrument, survey respondents responded to a series of items designed to assess their perceived susceptibility and severity to NoV infection. Either goodness-of-fit or ANOVA tests were performed to calculate weighted percentages of respondents' perceived susceptibility and level of concern associated with contracting NoV illness, being hospitalized, or dying from NoV infection.

Results: White/Non-Hispanic consumers were half as concerned about hospitalization from NoV compared to other races/ethnicities (12.58% White; 20.92% Black; 20.84% Other; 31.92% Hispanic, and 25.61% ≥2 races). Individuals with no internet access were significantly more concerned about dying from NoV infection (7%) compared to Internet users (1.23%). Consumers with infants, ages 0-1, reported greater concern about contracting an NoV infection (51.58%) than those with young children, ages 2-5 (33.12%).

Significance: The survey results will be used to guide development of consumer education materials that focus on NoV and that target specific population sub-groups. By analyzing the relationship between demographic characteristics and consumers' perceived susceptibility/severity associated with contracting NoV, food safety educators can develop more appropriate, evidence-based education materials that target specific audiences.

P2-158 Food Safety Information Recalled by Consumers Who Purchased and/or Received Raw Meat, Poultry, or Seafood Products from Online Purveyors

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Introduction: More than 550 US companies market fresh or frozen meat, fish, and/or seafood directly to consumers online, enabling consumers to have products delivered using common carriers such as FedEx and UPS. Due to the perishable nature of these products and their vulnerability to temperature abuse in transit, there is an increased potential for foodborne illness among consumers. Yet, many online food companies do not provide food safety information related to the receiving, handling, storing, or cooking of their products.

Purpose: The study characterizes the food safety information consumers recall having seen on company websites when purchasing fresh or frozen meat, fish or seafood products online, and in the packaging materials in the shipments of these products

Methods: A nationally representative sample of 1,002 US adults completed a Web-based survey. Within the prior year, all respondents had purchased or received gifts of perishable meat, fish, or seafood products ordered from online vendors that deliver using common carriers.

Results: The majority (68%) reported purchasing a perishable meat or seafood product online to be delivered to themselves or as gifts to others. Of those, only 34% recalled seeing any food safety information on the company's website; 24% recalled seeing instructions for storing the product after unpacking it. Most (90%) received a product delivered by a common carrier. Of those, 73% recalled seeing food safety information included in the product packaging; nearly two thirds (63%) of whom recalled seeing cooking temperatures for products, while 56% recalled seeing handling information, and 55% recalled seeing information about how to thaw frozen products.

Significance: The findings suggest that while some food safety information may be offered by online vendors of perishable meat, fish, and seafood products, there are still gaps in the information provided that may put consumers at risk for a foodborne illness.

P2-159 Food Thermometer Usage among Consumers Who Cook Raw Poultry: Results of a National Survey

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Introduction: *Salmonella* spp. and *Campylobacter* spp. are among the most common causes of foodborne disease in the United States annually. Most cases of salmonellosis and campylobacteriosis are associated with eating raw or undercooked poultry or eggs or cross-contamination. Several studies conclude 12-80% of foodborne illness is due to poor food handling practices in the home.

Purpose: To determine food thermometer use among U.S. consumers to check the doneness of poultry and to quantify barriers to thermometer use.

Methods: A total of 1,504 U.S. adults completed a nationally representative Web-based survey. All respondents had primary or shared responsibility for household grocery shopping and had prepared raw poultry and eggs in the past 30 days. A subset (n = 465) had prepared raw, ground poultry in the past 3 months.

Results: Sixty-two percent of consumers own a food thermometer. Of owners, the majority use a food thermometer to check the doneness of whole turkeys (73.2%) and whole chickens (56.7%). However, few consumers use one to measure the internal temperature of smaller cuts of poultry; 12-26%, depending on the cut or type of poultry. Several respondents reported, "I never thought to use one" when cooking smaller cuts of poultry (28.7%) or dishes made with ground poultry, such as meatloaf or patties (37.6%). Instead, respondents reported relying on other methods for determining doneness, like the internal color of meat (21-68%) or cooking time (46-57%). Almost half of all respondents reported they had little or no confidence they could "correctly use a food thermometer to determine if poultry is cooked to a safe temperature."

Significance: Evidence-based consumer education materials that can help reduce foodborne disease caused by *Salmonella* and *Campylobacter* are critically needed. Findings from this study will inform the development of a multifaceted, consumer intervention that includes interactive Web site, game, and mobile app and Extension educational curriculum.

P2-160 Quantifying Server Perceptions of Risk Communication Associated with Ordering Burgers in Restaurants

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Introduction: The FDA 2009 Food Code states that it is the duty of a restaurant to disclose the risk, and remind consumers, when ordering undercooked products such as ground beef. Currently, no food safety training is required for restaurant servers. *Escherichia coli* O157:H7 is estimated to cause approximately 35,000 illnesses per year associated with beef, while non-O157:H7 STECs cause approximately 45,000 illnesses per year associated with beef.

Purpose: The purpose of this study was to explore server knowledge and attitudes about risks associated with serving patrons undercooked hamburgers.

Methods: A semi-structured written survey was administered to servers (n = 56) at restaurants. Chain and privately owned restaurants were selected by compiling a list of burger restaurants in Wake County and calling restaurant managers to request participation in the survey, which was administered in person. Survey questions focused on methods used to measure doneness in hamburgers, beliefs around risks and server motivation to communicate risk to consumers.

Results: Overall, the majority of servers believe that medium well and well-done hamburgers are safer. 35% of private restaurant servers and 38% chain servers cite thermometer use as a safe method of measuring doneness. Color and amount of time cooked were also listed as acceptable indicators of doneness (private = 23% and chain restaurants =21%; private = 17% and chain restaurants = 25%, respectively). Over 35% of private servers say they never communicate risk to a customer while 55% of chain servers say they never communicate risk.

Significance: Although most servers believe that medium-well and well-done hamburgers are safer, this data indicates that the majority of restaurant servers rely on unreliable indicators of doneness when communicating with consumers. The data suggests that communicating risk is not highly valued by servers and shows the need for developing a food safety curriculum specifically geared towards improving risk communication between servers and consumers.

P2-161 Listeriosis Risk Factors among Two "At-risk" Consumer Groups: Pregnant Women and Older Adults

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Introduction: Sporadic incidence of listeriosis is frequently related to refrigerated ready-to-eat (RTE) food products stored and consumed in the domestic kitchen. Listeriosis is particularly associated with 'at-risk' consumers, predominantly affecting pregnant women and older adults. Consequently, such consumers should implement safe food handling/storage practices, including time and temperature control of RTE-foods. Changes in incidence identify foodborne illnesses. Determination of these consumers' cognitive and behavioural risk factors associated with listeriosis may identify association between perception and practices that influence domestic food-safety.

Purpose: The aim was to compare cognitive and behavioural risk factors of domestic food handling/storage practices among two 'at-risk' consumer groups.

Methods: Quantitative survey methods ascertained food-safety knowledge, self-reported practices, attitudes and perceptions of risk/control/responsibility during domestic food handling/storage. The study involved pregnant and post-partum (≤12 months) women (n=40) and older-adults (≥60 year) (n=100).

Results: Although no significant differences were determined ($P > 0.05$), knowledge, attitudes and self-reported practices regarding safe refrigeration temperatures and consuming opened RTE-foods within two days was better among pregnant women than older adults.

Conversely, knowledge of 'use-by' dates was significantly different ($P < 0.05$) with 95% of pregnant-women/72% of older adults knowing 'use-by' dates were the best indicator of food-safety. Pregnant women's attitudes towards 'use-by' dates were significantly more positive ($P < 0.001$) than older adults. Significantly fewer ($P < 0.05$) pregnant women (35%) reported to consume RTE-foods beyond the 'use-by' date than older adults (58%).

Significant correlations were determined between perceptions of risk, control and responsibility ($P < 0.05$) for older adults. However, pregnant women perceive the risk of foodborne illness to exceed their control.

Significance: Significant differences in knowledge, attitudes and self-reported practices may result from the variations in perceived risk, control and responsibility of food safety among these two groups of 'at-risk' consumers.

P2-162 Building the Business Case for Consumer Food Safety Education

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Introduction: How can consumer food safety education programming contribute to important business objectives of food retailers and food and consumer product companies? The Partnership for Food Safety Education set out to identify the most important benefits to business of connecting with customers and their own associates on the topic of safe food handling and home practices that can reduce risk of foodborne illness. The Partnership pursued this through a qualitative and quantitative research study.

Purpose: The purpose of the research was to identify evidence of how consumer safe food handling messages are perceived by customers/consumers and determine if promotion of such messages can be a winning loyalty-building strategy for food companies and retailers.

Methods: The research included two parts: 1) interviews with food industry executives; 2) a geo-demographically balanced online omnibus survey of more than 400 consumers. Telephone interviews lasting 30-45 minutes were conducted with 20 food safety and quality assurance executives, half in retail and half in food manufacturing. Using insight from Phase I, a detailed online questionnaire was designed and a sample of 400 grocery shoppers was surveyed. The sample was evenly split between males and females. A sample of 400 yielded results with 95% confidence level with a +/- 5 percent error.

Results: Information generated from manufacturers, retailers, and consumers confirm that affiliation with promoting the Fight BAC!® safe food handling messages is viewed favorably by consumers and aligns strongly with customers' trust in a company's quality, safety and integrity.

Significance: Being active in the promotion of home food safety messages is a winning strategy for food retailers and food manufacturers.

P2-163 Effect of Residual Sanitizers on *Salmonella enterica* Biofilm Formation

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Introduction: *Salmonella enterica* are a diverse group of bacteria that represent a serious risk to public health. Bacterial attachment on food and contact surfaces can lead to biofilm formation, and once in this state, bacteria are more resistant to sanitization and may serve as a continuous contamination source. Studies have shown that common sanitizers are not able to eradicate *Salmonella enterica* biofilms on food contact surfaces. Here we further explored this phenomenon by examining the effect of residual concentrations of common sanitizers on *Salmonella* biofilm formation on solid surfaces.

Purpose: To evaluate biofilm formation and sanitizer resistance of various *Salmonella* serotypes under different environmental conditions, and to investigate the effect of residual sanitizers on biofilm formation.

Methods: Sixty-four strains of four common *Salmonella enterica* serotypes isolated from cattle sources and from human cases of salmonellosis were tested for biofilm formation and sanitizer resistance under different environmental conditions using 96-well plate absorbance assays. Biofilm formation also was assessed after the addition of trace amounts of sanitizers to both planktonic cultures and pre-existing biofilms.

Results: Biofilm formation by various *Salmonella* serotypes was dependent upon specific cell surface structures and environmental conditions. Survival and recovery of biofilm cells after sanitization correlated with high biofilm mass that provided better protection. The presence of trace amounts of sanitizers enhanced biofilm formation of planktonic *Salmonella* cultures as well as increased bacterial colonization ($P < 0.05$) on pre-existing biofilms ($P < 0.05$). This was observed in certain *Salmonella* strains but not in all strains tested. These data suggest the existence of a unique genetic control mechanism responsible for this observation, which requires further investigation.

Significance: The observation that residual sanitizers could increase *Salmonella* biofilm formation highlights the importance of establishing proper sanitization and cleaning procedures in commercial meat plants.

P2-164 Development of a Simple, Sensitive, and Cost-effective PCR Method for Detection of *Salmonella* in Environmental Samples

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Introduction: *Salmonella* is one of the leading foodborne pathogens of public health concern, causing millions of foodborne illnesses worldwide. Polymerase chain reaction (PCR) is widely used in the routine detection of foodborne human pathogens; however, challenges remain in overcoming PCR inhibitors present in environmental sample matrices.

Purpose: The objective of this study was to develop a simple, rapid, sensitive, and cost-effective PCR method for detection of *Salmonella* in environmental samples.

Methods: Six DNA template preparation methods, including direct boiling, with or without pre-spin, multiple washing, and two commercial DNA extraction kits, were compared using pure overnight cultures of *Salmonella* and those spiked with background microflora from enriched soil, cattle feces, and pecan samples. The effect of different amplification facilitators such as, bovine serum albumin (BSA), polyvinylpyrrolidone (PVP), polyethylene glycol (PEG) and gelatin on PCR sensitivity was also evaluated.

Results: Pre-spin of sample matrices in combination with the addition of 0.4% BSA and 1% PVP in the PCR mix was the most simple, sensitive, and cost-effective PCR method for detection of *Salmonella*. In the presence of 3×10^9 CFU/ml background microorganisms in enriched soil or fecal samples, 40 CFU *Salmonella* per reaction can be detected. This sensitivity was similar to that of pure culture tested.

Significance: The developed method is simple, rapid, sensitive, and cost-effective, with broad applicability for processing large numbers of environmental samples for PCR detection of *Salmonella*.

P2-165 Using "Nutritional-shift" Trials to Develop Mechanistic Models of the Lag Phase of *Escherichia coli* K-12

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Introduction: The use of mathematical models is widely accepted in food microbiology. A continuing goal is development of more mechanistically-based models that better describe transitions between lag/exponential and exponential/stationary growth phases. Buchanan et al. (1997) proposed that the lag phase could be subdivided into an adjustment period when the cells alter their physiological state and a metabolic period when the cells complete activities related to the first division.

Purpose: The current study evaluated whether "nutritional-shift" trials could be used to distinguish and quantify the adjustment and metabolic periods of the lag phase of *Escherichia coli*.

Methods: BHI-grown *E. coli* K12 cells were cultured in tryptic soy broth without a carbohydrate source (TSB-G) to early stationary phase and then transferred to TSB-G and TSB + 0.5% lactose (TSB+L). Growth was followed by viable counts and absorbance, and growth curves fitted using a 2-phase linear model. Cultures were assayed for lactase activities using the AOAC ONPG method. The trials were run at 15, 20, 25, 30, 35, and 40°C.

Results: The growth kinetics of lag and log phases and their standard deviations were similar in both cultures, and displayed the expected temperature dependency. Lag times and specific growth rates were used to estimate the adjustment period value, which displayed a similar temperature dependency. Detectable lactase activity was limited to TSB+L cultures, with first appearance occurring slightly after the lag phase, indicating that the expression of lactase didn't occur until the completion of lag phase. Using the standard deviations from the trials it was possible to effectively describe the shape of the growth curve using Monte Carlo simulation software.

Significance: The use of nutritional-shift appears to be an effective tool for developing more mechanistic growth models, however, measurements of gene up-regulation are needed to better map the timing of the initial adjustment period.

P2-166 Free-living Amoebae as Reservoirs for the Transmission of Norovirus

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Developing Scientist Competition

Introduction: Human noroviruses (HuNoV) are the most common cause of foodborne disease outbreaks in the United States. The most common food commodities implicated in HuNoV outbreaks are leafy greens; however, the vehicle of transmission and point of contamination are often unknown. Here, we hypothesize that common free-living amoebae (FLA) ubiquitous in the environment may act as reservoirs of HuNoV and facilitate the transmission of these pathogens to fresh produce.

Purpose: To investigate FLA as reservoirs for HuNoV transmission the interaction between two *Acanthamoeba* species and two HuNoV surrogates were evaluated. The *Acanthamoeba* species include *A. castellanii* and *A. polyphaga*. In addition, two HuNoV surrogates – murine norovirus type 1 (MNV-1) and feline calicivirus (FCV) were evaluated and compared in this study.

Methods: Initially, 3×10^6 amoebae were inoculated with 3×10^6 PFU of each virus and incubated at 25°C for 1 hour. Amoebae were washed 3 times with 1×PBS to remove residual viruses followed by resuspension in 1 ml amoeba culture medium. Virus titers were then analyzed by plaque assay to determine the number of virus associated with amoebae. Also, amoeba growth curves at 25°C were established after virus inoculation.

Results: After amoeba-virus incubation, 490 PFU and 337 PFU of MNV-1 were associated with *A. castellanii* and *A. polyphaga*, respectively, via plaque assay while only few or none FCV were detected. Moreover, the growth curves of amoebae with and without virus inoculation were not statistically significantly different. To clarify the interaction between amoebae and HuNoV surrogates, further experiments including localization and longitudinal analyses of viruses in amoebae are underway.

Significance: The findings of this study indicate that MNV-1 associate with *A. castellanii* and *A. polyphaga* without affecting the normal growth of these amoebae. This is significant in understanding the HuNoV survival in the environment and the future control of HuNoV outbreaks.

P2-167 A Microbial Stability Calculator for Beverage Formulations

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Poster

Introduction: In order to develop a reliable predictive model of microbial stability for different beverage formulations, specific inputs must be known. These include the concentrations of preservatives used in product development as well as intrinsic characteristics such as the pH level. It is possible to integrate these data sources into a system for the statistical modelling of microbial stability.

Purpose: To develop models and software to examine the effects of different input parameters such as preservatives, food additives, carbonation and pH on the microbial stability of beverage formulations.

Methods: A database of experimental data involving 659 beverage formulations each characterized by seven explanatory variables was used to develop two separate models. Since the outcome variable is binary (product is either stable or unstable) a logistic regression model with interaction terms was fitted to the data. Various statistical methods and tests were used in order to select and validate the models including deviance, sensitivity and specificity, detection of outliers and influential observations.

Results: It was found that dividing the experimental data into two categories, carbonated and non-carbonated, and constructing two separate models for each case gave the best results in terms of optimizing the accuracy of predictions, giving an sensitivities of 85% and 82%, respectively. Given appropriate input parameters within the experimental range, the models predict whether a product is stable or not and outputs the probability associated with the prediction. The developed and validated models were integrated into a web-based software system which can be used for routine stability assessment.

Significance: The software tool presents a rational and realistic methodology for assessing the microbial stability of beverage formulations. This helps the food manufacturers to more efficiently exploit the effects of changing input parameters on the stability and safety of their products without the requirement of performing as many challenge tests.

P2-168 Estimation of *Bacillus stearothermophilus* Kinetic Parameters of Inactivation Using a Sub-lethal Adaptation Model in Conduction-heated Foods

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Introduction: Estimation of inactivation kinetic parameters for spores is typically done using isothermal experiments with rapid come-up times in small samples of uniform laboratory media. These parameters are then used to predict log reductions of spores in commercial conditions that may be very different from those in the isothermal simulations. Some isothermal studies have shown that spores may become more resistant to inactivation as they spend more time in the sub-lethal temperature range.

Purpose: The purpose of this study was to determine if the inactivation kinetics of a spore under commercial thermal processing conditions was better represented by a sub-lethal adaptation model than by the first-order or Weibull models.

Methods: Previous dynamic data were used. Cans (6 cm dia., 3.48 cm height) were filled with pea puree inoculated with *Bacillus stearothermophilus* ATCC 7953 spores at ~9.5×10^5 CFU/ml. The cans were heated in duplicate at three different steam temperatures (104.4, 112.8, 120.6°C) for heating times from 21 to 305 min, for a total of 24 samples. Each can's mass-average spore concentration was both enumerated using a non-selective media and incubation at 55°C, and computed using three different models. Parameters were estimated using an inverse method.

Results: Log-reductions ranged up to 2.5. The first-order model performance was poor, as shown by a 0.24 log CFU/ml RMSE and AICc = -62.8. The Weibull model was significantly better, with RMSE = 0.12 log CFU/ml, AICc = -95.6. The best model was the Weibull with sub-lethal term: RMSE = 0.098 log CFU/ml, AICc = -100.6. The parameter errors for d105°C = 3.2%, for z = 2.8%, and for n = 9.4%.

Significance: The results of this work indicate that for thermally-processed foods with different heating rates, sub-lethal history may affect prediction of survivors. Process designers and researchers may consider using experiments more representative of commercial processes.

P2-169 Evaluation of a Hot-Fill-Hold Process: Effects on Accumulated Lethality on the Underside of the Lid and Vacuum Formation

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Introduction: The hot-fill-hold process is commonly used for processing acid and acidified foods. The product is heated, filled into containers, closed and kept hot for a specified time. Packing materials are heated by the contact with the hot product. Currently, there are no processing guidelines or published data indicating temperatures and times required to achieve proper thermal processing of the packing materials and adequate vacuum to prevent spoilage.

Purpose: We evaluated a hot-fill-hold process by assessing the effect of process conditions on accumulated lethality values measured on the underside of the lid and vacuum formation, and modeled the conditions' effects.

Methods: Sucrose solutions (10 to 50°Brix) were prepared and heated (79.4 to 96.1°C). Glass canning jars (473 and 946 ml) were used, with two lid widths (regular and wide). After hot-filling, the temperature on the underside of the lid (coldest point in the container) was measured for 5 min using a surface thermocouple. After cooling at room temperature container vacuum was measured. A full factorial design was used to test the effects of sucrose concentration, filling temperature, jar size and lid width on the logarithmic transformation of accumulated lethality and vacuum formed. Multiple linear regression analysis was performed and significant ($P < 0.001$) terms of the model selected. Experiments were conducted in triplicate.

Results: Regression analyses ($R^2 > 0.98$) showed that all three- and four-way, and most two-way interactions were not significant in both models. Sorted parameter estimates showed that the solution's temperature and jar size had the most significant effect on the responses lethality and vacuum, respectively. Overall, lethality values ranged between 0.001 and 0.1 min (T_{ref} 93.3°C, z-value 8.9°C), and vacuum between 20 and 60 kPa.

Significance: These results contribute to the establishment of processing guidelines ensuring production of safe and stable products with optimized processing temperatures and times to enhance quality of hot-fill-hold products.

P2-170 Bacterial Diversity in Laboratory Heat-treated and Commercially Pasteurized Fluid Milk Along the Milk Chain

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Introduction: The dairy industry needs to control spoilage microorganisms, such as spore-forming bacteria (*Bacillus* spp. and related genera) and Post-Pasteurization Contaminants (PPC), to improve the quality and shelf life of fluid milk. PPC can be controlled with pasteurization and sanitization procedures, but spore-forming bacteria can survive pasteurization and persist in the plant environment by forming biofilms. Strategies to better control these organisms require an assessment of the bacterial ecology throughout the fluid milk chain.

Purpose: Evaluate the bacterial diversity of laboratory heat-treated and commercially pasteurized fluid milk along the milk chain, and identify common genera present in both set of samples.

Methods: Raw and pasteurized milk samples were collected at different locations (farm, trucks, processing plant, and packaged products) from a medium-size milk chain in the Midwest during three periods (Spring-2012, Fall-2012 and Spring-2013). All raw milk samples and aliquots of some pasteurized milk samples (Spring-2013) were heat-treated in the laboratory (80°C, 12 minutes) to eliminate vegetative cells. Milk samples (heat-treated and commercially pasteurized) were stored at <7°C for 21 days. Bacterial isolates were collected from samples at different time points and characterized using *rpoB* and/or 16S rRNA sequencing.

Results: A total of 220 bacterial isolates were collected from all milk samples. The isolates from laboratory heat-treated milk samples were identified as *Bacillus* spp. (79%, 109/138), *Paenibacillus* spp. (14%, 20/138) and other spore-forming bacteria (7%, 9/138); while the ones from commercially pasteurized milk samples were characterized as *Bacillus* spp. (56%, 46/82), PPC (28%, 23/82), *Paenibacillus* spp. (11%, 9/82), and other spore-forming bacteria (5%, 4/82).

Significance: Although most of the isolates were identified as spore-forming bacteria, PPC is still a concern in commercially pasteurized milk, especially during the filling stage. Spore-forming bacteria were detected in samples from different locations, thus strategies for control would need to encompass the entire milk chain.

P2-171 Developing Environmental Pathogen Monitoring Programs for Small Dairy Processors: A Proactive Approach to Dairy Food Safety

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Developing Scientist Competition

Introduction: While environmental pathogen monitoring programs in processing plants are critical for assuring food safety, many smaller processors do not necessarily have resources needed to develop, implement, and validate effective programs.

Purpose: This project was designed to implement and validate environmental pathogen monitoring programs for *Listeria monocytogenes* and *Salmonella* in small dairy processing facilities.

Methods: Environmental sampling was performed at five cheese production facilities throughout New York State. Samples were analyzed by plating on selective media and polymerase chain reaction. The obtained pathogen isolates were further characterized by pulsed field gel electrophoresis (PFGE). Corrective action was suggested according to the results.

Results: A total of 1346 samples were collected. Of these, 34 (2.52%) tested positive for *Listeria* spp. and 35 (2.60%) were positive for *L. monocytogenes*. The prevalence of *Listeria* in a given plant varied from <1% (no *Listeria* detection) to 6.6%, whereas *Salmonella* was only detected in a single plant. While PFGE patterns suggest persistence of some *L. monocytogenes* strains in the processing environments, presence of multiple PFGE patterns in most plants suggested multiple contamination sources. Independent validation sampling (i.e., collection of samples from sites not routinely sampled) found similar *L. monocytogenes* prevalences as routine sampling, suggesting effective sampling plans.

Significance: This study provides a model for development and implementation of effective environmental pathogen monitoring programs. Importantly, we also present a procedure for validation of sampling plans through independent sampling from environmental sites not included in the routine sampling plans.

P2-172 Identification of Farm Management Practices Associated with the Presence of Psychrotolerant Sporeforming Bacteria in Bulk Tank Milk

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Developing Scientist Competition

Introduction: Shelf lives of high quality pasteurized fluid milk products are limited by outgrowth of psychrotolerant sporeforming bacteria (e.g., *Bacillus* spp. and *Paenibacillus* spp.) which are commonly found in farm environments and raw milk. Certain strains not only survive pasteurization but are capable of growing at refrigeration temperatures, thereby causing pasteurized fluid milk spoilage.

Purpose: To evaluate possible associations between on-farm management practices and levels of psychrotolerant sporeformers in bulk tank milk.

Methods: One bulk tank sample was obtained and a management/herd health questionnaire was administered to 99 New York State dairy farms. Milk samples were spore pasteurized (SP) (80°C (176°F), 12 min) and analyzed for most probable number and for sporeformer counts on initial day of SP, and after refrigerated storage (6°C) at 7, 14, and 21 d post-SP. Management practices were analyzed for association with sporeformer counts and bulk tank somatic cell counts, respectively. Select isolates were characterized by partial *rpoB* gene amplification, sequencing, and allelic type assignment.

Results: Sixty-two farms had 'high' sporeformer growth (≥ 3 log CFU/ml at any day post-SP) with an average sporeformer count of 5.20 ± 1.41 mean log CFU/ml at 21 d post-SP. Thirty-seven farms had 'low' sporeformer numbers (< 3 log CFU/ml for all days post-SP) with an average sporeformer count of 0.75 ± 0.94 mean log CFU/ml at 21d post-SP. The percent of milking cows with dirty udders in the milking parlor and herd size were significantly associated with farms that had 'high' sporeformer growth. The majority of isolates characterized represented either *Bacillus* spp. (71.4%; 317/44) or *Paenibacillus* spp. (26.4%; 117/444).

Significance: On-farm adjustments in management decisions specifically focused on udder cleanliness may directly impact the shelf-life of pasteurized fluid milk.

P2-173 Characterization of Bacterial Communities during the Production of Bola Cheese by Pyrosequencing

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Developing Scientist Competition

Poster

Introduction: Mexican artisanal cheese made with raw milk such as Bola cheese, is considered a high-risk product. Pasteurization eliminates pathogens from raw milk, but also microorganisms that produce desired sensorial characteristics. By knowing the dynamics of microbial communities during a traditional cheese production process, this could help to detect bacteria that can be used as starter cultures to produce desirable sensorial characteristics in cheese produced with pasteurized milk.

Purpose: To characterize bacterial communities through the Bola cheese production process and to identify bacteria that could be used as starter cultures, by pyrosequencing analysis.

Methods: A total of 24 samples consisting of milk, curd and cheese ripened for 50 and 110 days were obtained during dry and rainy season from 3 producers of Bola cheese. Total DNA was extracted from samples and sent to Molecular Research DNA Laboratory (Shallowater, TX) for DNA sequencing of 16S region amplified using primers 27Fmod (5'-AGRGTTTGATMTGGCTCAG-3') and 530R (CCGCNGCNGCTGGCAC). Pyrosequencing was performed with Roche 454 FLX titanium instruments and reagents. The sequence database was processed using Mothur software.

Results: Dairy samples collected from each producer in both seasons had a different bacteria composition. No pathogenic bacteria were found in any step of the process. Predominant genera in raw milk consisted in *Streptococcus, Lactococcus, Lactobacillus, Macrococcus* and *Staphylococcus*; also environmental microorganisms were present. In curd and cheeses, the predominant species were *Streptococcus thermophilus, Lactococcus lactis, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus delbrueckii* and *Enterococcus faecium*.

Significance: It was possible to identify bacteria that could be used as a starter culture, which in combination with pasteurized milk in production of Bola cheese could generate high quality and safe products with desirable sensorial characteristics. The information generated in this work would be useful for producers of Bola cheese, by helping them standardize the production process, in establishing provenance, and to preserve a traditional food that supports the economy of local producers and their families.

P2-174 Molecular Typing and Probiotic Attributes of *Lactobacillus casei* Isolated from Fermented Goat Cheese (Anbaris)

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Introduction: Anbaris is a traditional dairy product prepared by naturally fermenting raw goat's milk for a period of 2-6 months. The fermentation process is solely dependent on the naturally occurring Lactic Acid Bacteria (LAB) that yields a highly acidic (pH 3.14) product with an extended shelf life and good organoleptic properties.

Purpose: This study aimed at identifying the isolated LAB strains and assesses their potential probiotic characteristics, their anti-microbial susceptibility and anti-bacterial activity.

Methods: Samples of Anbaris were obtained from two rural regions in Lebanon (Kweikh and Aarsal) and analyzed for the presence of Lactic Acid Bacteria. Suspected colonies were confirmed by API 50 system and further identified by PCR and Pulsed-Field Gel Electrophoresis (PFGE). The disc-diffusion method was used to assess the anti-microbial susceptibility. The isolates of LAB were also tested to determine their potential probiotic characteristics by temperature and salt tolerance, NH_3 production, citrate utilization, acid and bile tolerance, Angiotensin Converting Enzyme (ACE) inhibitory activity and antibacterial activity against *Staphylococcus aureus.*

Results: Six isolates showed probiotics attributes with their ability to grow at different salt and acid concentrations and tolerance to acid and bile. They had Angiotensin Converting Enzyme (ACE) inhibitory activity and antibacterial activity against *Staphylococcus aureus*. The isolates were further identified by PCR profiling and API 50 system as strains of *Lactobacillus casei*. The disc-diffusion method showed them resistant to at least 8 antibiotics and the PFGE results indicated a 45% similarity between the isolates although they were obtained from two different regions.

Significance: This study revealed a highly nutritious traditional product naturally supplemented with beneficial probiotics that are possibly new strains of *Lactobacillus casei*. They can be used in industrial applications to prevent disease and improve gastrointestinal health in developing countries.

P2-175 Microbiological Characterization of the Manufacturing Process of Artisanal Chihuahua Cheese

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Introduction: Mexican artisanal Chihuahua cheese is a regional product made from raw or pasteurized cow´s milk by menonites in Chihuahua State (Mexico). Due to the quality, characteristics and native microorganisms of the milk used, the properties of the final product may change. It has been suggested that the season in which the cheese is elaborated may influence the microbiological dynamic of manufacturing process of this product.

Purpose: The aim of this work was to evaluate the dynamic and diversity of microorganisms during the manufacture of artisanal Chihuahua cheese of raw milk in three different sampling times during a year.

Methods: Samples of raw milk, curd, curd cutting and cheese were obtained of three cheese factories at Chihuahua State (Mexico). Three samplings were performed during a year (autumn, winter and summer). Microbiological analysis to determinate coliforms, aerobic mesophilic, molds, yeast, *S. aureus* and *Enterococcus* sp. were done in raw milk and cheese and mesophilic and thermophilic lactobacilli species, *Lactococcus* sp., thermophilic coccus and *Enterococcus* sp. in all samples (processing and final product).

Results: There were statistically significant differences ($P < 0.05$) between sampling seasons in the microbiological analysis in all of the microorganisms determined in samples of the cheese factory A; in factory B there wasn´t significant difference ($P = 0156$) in the case of yeast; and in factory C there weren´t significant difference in coliforms ($P = 0.805$), aerobic mesophilic ($P = 0.438$) and mesophilic lactobacilli ($P = 0.210$). In the three cases, the media analysis by Tukey showed that the counts of some of the determined microorganisms are equal in two sampling seasons.

Significance: The results suggest that there could be an influence of the season in which the cheese is made, but also the manufacturing practices of each cheese factory could be playing an important role in determining the dynamic of the microorganisms presents.

P2-176 Growth of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, and *Staphylococcus aureus* on Cheeses during Extended Storage at 25°C

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Introduction: The US Food and Drug Administration considers types of cheese with pH >4.2 and a_w >0.92 to be potentially hazardous. Without a product assessment showing no pathogen growth, most cheeses could therefore not be stored for extended time at retail under non-refrigerated conditions.

Purpose: To assess the compositional factors that affect pathogen growth on cheese at non-refrigerated conditions.

Methods: We tested 67 natural market cheeses for their ability to support growth of *Listeria monocytogenes* (LM), *Salmonella* (SALM), *Escherichia coli* O157:H7 (EC), and *Staphylococcus aureus* (SA) during 15-days at 25°C. Hard (Asiago and Cheddar), semi-hard (Colby and Havarti), and soft cheeses (Mozzarella and Hispanic-style) were among types tested, and included regular, reduced-sodium, and reduced-fat types. Single-pathogen cocktails were prepared and individually inoculated onto cheese slices (~10^5 CFU/g). Cocktails were comprised of 10 strains of LM, six of SALM, or five of EC or SA. Inoculated slices were packaged and stored at 25°C for < 15 days, with surviving inocula enumerated every three days. Moisture (%), salt (%), titratable acidity (%), pH, a_w, and lactic acid bacterial (LAB) count were measured.

Results: Pathogens did not grow on 44 cheeses over the 15 days, while 13 cheeses supported growth of SA, six of SALM, four of LM, and three of EC. Of the cheeses which supported pathogen growth, all supported *S. aureus* growth, ranging from 0.62 to 3.08 log CFU/g (avg. 1.70 log CFU/g). Growth of *Listeria monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 ranged from 0.60-2.68 (avg. 1.60 CFU/g), 1.01 to 3.02 log CFU/g (avg. 2.05 log CFU/g), and 0.41 to 2.90 log CFU/g (avg. 1.69 CFU/g), respectively. Cheeses which supported pathogen growth also supported LAB growth.

Significance: Cheese pH at time 0 and salt-in-the-moisture-phase (%SMP) were compositional factors most affecting pathogen growth, with pH having a dominant effect. Except for certain surface- and mold-ripened cheeses, or Swiss-style cheeses, pathogen growth on cheeses made with cows' milk was correlated with initial pH and %SMP.

P2-177 Effect of Sodium Reduction on *Listeria monocytogenes* in Mozzarella Cheese Stored at 4 and 12°C

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Introduction: Salt (NaCl) is added to foods to help inhibit microbial growth and improve flavor. However, a diet high in sodium has been associated with high blood pressure, leading to an increased risk for cardiovascular disease and stroke. While lowering the amount of salt in processed food products could decrease these risks, the microbial stability of reduced sodium products may be altered.

Purpose: The purpose of this study was to determine the fate of *Listeria monocytogenes* in artificially inoculated full sodium and low sodium mozzarella cheese stored at 4 and 12ºC.

Methods: Low sodium (0.65% NaCl) and full sodium (1.80% NaCl) Mozzarella cheese were cut into 15 g pieces and inoculated with approximately 2 log CFU/g of a *L. monocytogenes* cocktail. The Mozzarella cheese was tested for *L. monocytogenes,* coliforms, yeast, mold, psychrotrophs, pH and water activity after 0, 1, 7, 15, 35, 60 and 90 d and after 0, 1, 3, 7, 15 and 35 d when stored at 4 and 12°C, respectively. Counts were compared using ANOVA.

Results: The initial water activities were 0.9854 ± 0.0036 and 0.9796 ± 0.0089 for the low and full sodium Mozzarella cheeses, respectively. There was no statistical difference ($P < 0.05$) in the pH or in the recovery of *L. monocytogenes,* coliforms, yeast, mold or psychrotrophs between the full and low sodium Mozzarella cheese at both 4 and 12°C. Under all test conditions, *L. monocytogenes* decreased by an average of 0.62 ± 0.35 log CFU/g at the end of incubation.

Significance: These data suggest that sodium could be reduced in Mozzarella cheese without altering the microbial stability.

P2-178 Microbiological Assessment and Intervention to Mitigate Environmental Contamination and *Listeria monocytogenes* Risk in Artisan Cheese Facilities

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Introduction: In response to concerns that artisan and farmstead cheese operations may present higher food safety risks, the U.S. Food and Drug Administration has increased inspections of these small-scale producers in recent years. Concern over the safety of soft and semi-soft cheese has also resulted in focused environmental sampling for *Listeria monocytogenes* across the industry.

Purpose: As an expansion of a previously successful program, the Vermont Institute for Artisan Cheese provided one-on-one technical assistance to 9 Vermont cheese makers through targeted, comprehensive audits and microbiological data collection to identify and mitigate risks specific to each business.

Methods: Sponge samples collected from target areas based on facility conditions and traffic patterns were analyzed for aerobic bacteria (n = 89), coliforms (n = 150), and *Listeria* species (n = 268). **Results** were used to develop risk mitigation strategies. Samples of raw milk, curds, whey, brine, and finished cheese were collected and screened for *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 and *Staphylococcus aureus*. Follow up visits were conducted to assess the implementation of recommendations and to collect samples for follow up analysis.

Results: In addition to reduction in aerobic bacteria and coliforms, *Listeria* spp. and *L. monocytogenes* contamination rates for non-food contact surfaces decreased from 18.2% to 6.3%, and 4.9% to 2.5%, respectively. *Staph. aureus* was the most common organism isolated from in-process samples and brine although *L. monocytogenes* was detected in the milk, whey and resulting cheese from one farm on both visits which was preliminarily traced back to contaminated feed. No *E. coli* O157:H7 or *Salmonella* were detected in any sample.

Significance: Our data confirm the value of this type of program as an effective tool to help businesses better understand and manage food safety risks and validate preventive controls, with declines in incidence and levels of target pathogens and indicator organisms between visits.

P2-179 Mathematical Model to Describe the Kinetic Behavior of *Escherichia coli* in Mozzarella and Cheddar Slice Cheeses under Constant and Dynamic Temperatures

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Introduction: *Escherichia coli* foodborne outbreaks have been linked to consumption of processed cheeses, but the kinetic behavior of *E. coli* in processed cheese has not been studied.

Purpose: Therefore, the objective of this study was to develop a predictive model to describe the kinetic behavior of *E. coli* in Mozzarella and Cheddar slice cheeses under constant and dynamic temperature conditions.

Methods: A five-strain mixture of *E. coli* was inoculated on Mozzarella slice and Cheddar slice cheeses at 3-4 log CFU/g, and the inoculated samples were aerobically stored at 4-30°C for 24-1,320 h. Total bacteria and *E. coli* cell counts were then enumerated on tryptic soy agar and MacConkey sorbitol agar, respectively. The Baranyi model was fitted to the growth data of *E. coli* to calculate maximum specific growth rate (μ_{max}; log CFU/g/h), lag phase duration (*LPD*; h), lower asymptote (log CFU/g), and upper asymptote (log CFU/g). The square root model or a polynomial equation was fitted to the kinetic parameters to describe the effect of storage temperature on the kinetic parameters. Dynamic models were also developed to describe kinetic behavior of *E. coli* at dynamic temperature. The performance of the developed model was evaluated by root mean square error (*RMSE*).

Results: The μ_{max} values increased ($P < 0.05$), but *LPD* values decreased as temperature increased. At 4°C, *E. coli* cell growth was not observed for both cheeses. μ_{max} and *LPD* values were different between Mozzarella cheese (μ_{max}: 0.01-0.33 log CFU/g/h; *LPD*: 1.79-30.44 h) and Cheddar cheese (μ_{max}: 0.00-0.28 log CFU/g/h; *LPD*: 6.08-768.00 h). For Mozzarella cheese, *E. coli* grew at 10-30°C with μ_{max} of 0.01-0.33 log CFU/g/h, but *E. coli* grew at 15-30°C with μ_{max} of 0.03-0.28 log CFU/g/h in Cheddar cheese. In addition, developed dynamic models described appropriately kinetic behavior of *E. coli*. *RMSE*(0.176-0.337) indicated that the developed models were appropriate.

Significance: The developed models in this study could be useful in describing the kinetic behavior of *E. coli* in Mozzarella and Cheddar cheeses under constant and dynamic temperature.

P2-180 Kinetic Behavior of *Escherichia coli* on Natural Cheeses under Dynamic Temperature Condition

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Introduction: *Escherichia coli* have been isolated from cheeses, which are usually stored at dynamic temperature, but mathematical models to control pathogens in cheese have been usually developed for constant temperature.

Purpose: This study developed mathematical models to describe kinetic behavior of *Escherichia coli* in natural cheese during storage at constant and dynamic temperatures.

Methods: A five-strain mixture of *E. coli* was inoculated on 15 g of natural cheeses (Brie and Camembert cheeses). The samples were then aerobically stored at 4-30°C for 1-55 days under constant temperature and dynamic temperature (4-14°C). Total bacterial (tryptic soy agar) and *E. coli* (MacConkey sorbitol agar) cell counts were enumerated. The Baranyi model was fitted to the growth data of *E. coli* to estimate maximum specific growth rate (μ_{max}) and lag phase duration (*LPD*). The μ_{max} and *LPD* were further analyzed with the square root model and linear equation, respectively. Moreover, dynamic models were developed with changing temperature profile. Subsequently, the performance of the developed models was evaluated by the root mean square error (*RMSE*).

Results: Obvious growth of *E. coli* was observed in Brie and Camembert cheeses at 10-30°C. μ_{max} increased ($P < 0.05$) up to 0.94 log CFU/g/h, but *LPD* decreased ($P < 0.05$) from 24.49 to 5.40h as temperature increased. No differences of μ_{max} and *LPD* were observed between Brie (μ_{max}: 0.03-0.94 log CFU/g/h; *LPD*: 5.40-20.13h) and Camembert cheeses (μ_{max}: 0.03-1.03 log CFU/g/h; *LPD*: 5.92-24.49h). The secondary model properly described the effect of storage temperature on the parameters with high R^2 (0.890-0.984). In addition, developed dynamic models were appropriate to describe the kinetic behavior of *E. coli.* The developed models showed appropriate prediction results with 0.218-0.264 of *RMSE*.

Significance: The developed mathematical models in this study should be useful in describing kinetic behavior of *E. coli* in natural cheeses under various storage conditions.

P2-181 Withdrawn

P2-182 Ochratoxin A in Naturally Stored Barley, Durum and Hard Red Spring Wheat

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Introduction: Ochratoxin A (OTA) is a mycotoxin of significant health concern that is present in a variety of foods and cereal grains around the world. OTA contamination can be controlled through the implementation of proper storage practices. Cereal grains in the supply chain must be surveyed in order to gain a holistic understanding of OTA occurrence after storage and prior to processing.

Purpose: The purpose of this study was to determine the prevalence and level of OTA in barley, durum and hard red spring (HRS) wheat grown in the Northern Great Plains and northwest regions of the United States and stored under natural conditions.

Methods: Barley (n = 60), durum (n = 58) and HRS (n = 144) wheat samples (~1 kg) that had been stored for < 1-16 months were collected over two years by multiple commercial grain companies. Samples were analyzed for OTA using high-performance liquid chromatography with fluorescence detection. The binomial proportions obtained for each grain type were tested for significance between harvest years with the Chi-square test using SAS software.

Results: OTA was detected in 32 (12.2%) of the samples and of those samples 26 (81.3%) had been stored for ≥ 6 months. One barley and four wheat samples exceeded the European Union limit of 5.0 ng/g OTA. In 2012, the number of OTA positive durum and HRS samples decreased but barley increased as compared to 2011; a significant ($P < 0.05$) difference in proportions between years for HRS only.

Significance: To our knowledge this is the first study in the United States that targets OTA in barley and wheat stored under natural conditions. The information garnered in this study provides valuable insight as to the annual variation that occurs in the cereal grain supply as well as the actual OTA levels entering the food chain which, ultimately, reach the consumer.

P2-183 Mycotoxin Occurrence in Three Types of Forages (Alfalfa, Sorghum and Grass) Distributed in Nuevo León, México

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Introduction: Mycotoxins are secondary metabolites produced by specific filamentous fungi that are common contaminants of agricultural commodities. These compounds are toxic to human and animals, and are a health problem worldwide. Mycotoxin-containing feed can cause serious diseases in farm animals and can cause substantial economic losses. Scientific information about the prevalence of mycotoxin in cattle feed is very limited in Mexico.

Purpose: The objective of this study was to determine and quantify the occurrence of six mycotoxins in three different types of forage distributed in the state of Nuevo Leon, Mexico.

Methods: The analyses were carried out in 120 samples of alfalfa, sorghum and grass used for animal consumption from farms and commercial locations in the state of Nuevo León Mexico during the 2013-2014 period. The presence of total aflatoxins, deoxynivalenol, fumonisins, ochratoxin, T-2 toxin and zearalenone was determined in all the samples by (ELISA) using Neogen Veratox mycotoxin test kits.

Results: All forages types exhibited mycotoxin content although significant differences ($P > 0.05$) were found between these. Alfalfa forages exhibited higher content of aflatoxins, deoxynivalenol, fumonisins, ochratoxin, T-2 toxin and zearalenone (average of 1.45, 0.38, 0.17, 76.68, 9.89 and 214.54 ppm, respectively) compared to the other forages. Levels of mycotoxins in alfalfa forages were higher (average of 75 and 84% of aflatoxins, 90 and 66% of deoxynivalenol, 32% of fumonisin, 48 and 89 % of ochratoxin, 58 and 76 % of T-2 toxin and 19 and 69% of zearalenone), compared to the levels found in sorghum and grass, respectively.

Significance: This study generated data about the status of mycotoxin contamination of these feeds in this region, and provide information to establish appropriate control measures to reduce the risk of contamination of feeds, particularly when used in mixtures with other type of forage susceptible to fungal contamination.

P2-184 Potassium Dichromate Toxicity: Protective Effect of Methanolic Extract of *Cochrorus olitorus* in Albino Rats

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Developing Scientist Competition

Introduction: Hexavalent chromate compounds are well known human carcinogens. In addition to other compounds, they pose health hazard in several part of the world especially in developing countries. Exposure to the compounds is known to induce several cancers of the respiratory system and internal organs and there is currently no known dependable treatment.

Purpose: The aim of the study is to evaluate the safety of the leafy vegetable and herb, *Cochrorus oitorus* (CO*)* and its usefulness against $K_2Cr_20_7$ toxicities.

Methods: Negative control animals were fed distilled water, while the positive control rats received $K_2Cr_20_7$ on 7th, 14th, 21st, 28th, 35th and 42nd day. Test rats were exposed to 25, 50 and 100 mg/kg body weight of CO alone for 42 days and/or 12 mg/kg body wt of $K_2Cr_20_7$ on the 7th, 14th, 21st, 28th, 35th and 42nd day of the experiment before the animals were sacrificed. The frequency of micronucleated polychromatic erythrocytes (mPCEs) was monitored in bone marrow cells while aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine levels were assessed in the serum. Haematological parameters were also monitored in test and control animals. The phytochemical analysis of CO was also investigated.

Results: $K_2Cr_2O_7$ significantly ($P < 0.05$) increased the levels of mPCE, AST, ALT, creatine, total white blood cells and lymphocytes when compared with the control, while percentage pack cell volume and neutrophils were reduced. In contrast, treatment with the different doses of CO restored the markers towards the levels of the control. Extract of CO is rich in flavonoids, saponins, antraquinnones, terpernoid and phenols which may be responsible for the protection observed in this study.

Significance: Our results therefore suggest that methanolic extract of CO possesses a promising potential in the treatment/management of chromate toxicity.

P3-01 Antibiotic and Disinfectant Resistance of *Escherichia coli* Isolated from Pork in Sichuan Province, China

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Introduction: Retail meats are commonly contaminated with *Escherichia coli*. Antibiotic resistance rates in *E. coli* are rapidly rising. Wide use and misuse of quaternary ammonium compounds (QACs) in food environments may contribute to the emergence of disinfectant-resistant microbes. Limited information is available regarding the QAC resistance genes in *E. coli* isolates from pork in Sichuan province.

Purpose: The objective of this study was to determine the prevalence of QAC resistance genes and acquire information about the association of their presence and resistance to QACs and other antimicrobials in *E. coli*.

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Methods: 348 samples of pork were collected, and *E. coli* was isolated using selective mediums and confirmed by VITEK. Isolates were tested for sensitivity to 11 different antibiotics using the standard disk diffusion method. MICs of QACs were determined by the agar dilution method. The presence of 10 QAC resistance genes (*qacE, qacEΔ1, qacF, qacG, emrE, sugE(c), sugE(p), mdfA and ydgE/F*) were determined by PCR.

Results: 255 *E. coli* were obtained, and the resistance frequency to antibiotics were as follows: SMZ (61.57%), TET (61.18%), AMP (48.24%), KF (29.80%), KAN (22.35%), STR (21.18%), CIP (14.51%), CN (11.37%), CRO (6.67%), SAM (5.88%), and CAZ (2.75%). The MICs of BC, DDAC, CTAB, and CPC to *E. coli* were 16-1024 µg/ml, 4-1024 µg/ml, 16-512 µg/ml and 8-512 µg/ml, respectively. *sugE*(c) (83.14%) was the most prevalent QAC resistance gene, followed by *emrE* (50.98%), *mdfA* (55.69%) *ydgE* (53.73 %), *ydgF* (52.94 %), *qacF* (14.9 %), *qacEΔ1*(13.33%), and *sugE(p)* (5.88%). *qacE* and *qacG* were not detected in any of the isolates. A significant correlation was found between the presence of *qacEΔ1*, *qacF* and antibiotic-resistance ($P < 0.05$).

Significance: *E. coli* might be significant in the spreading of antibiotic and QAC resistance in meats. The use of QACs in food production may have been partially responsible for an increase in QAC-resistant bacteria.

P3-02 Influence of Novel Chemical Compounds on Virulence Gene Expression by Shiga Toxin-producing *Escherichia coli*

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Developing Scientist Competition

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens that can cause hemorrhagic colitis and hemolytic uremic syndrome. The ability to produce Shiga toxin is controlled by the expression of virulence factor genes. We have identified a class of low molecular weight chemical compounds (LMWC) capable of inhibiting Shiga toxin production, which could diminish the virulence of STEC.

Purpose: This study was conducted to determine the influence of LMWC on the expression of the Shiga toxin genes, *stx1* and *stx2*, and the intimin gene, *eaeA*, by STEC.

Methods: STEC strains were grown in tryptic soy broth containing 0.1% DMSO (control) and 100 µm LMWC at 37°C for 20h, and total RNA was extracted for producing the cDNA templates for PCR. Primers were designed for *16S* (reference), *hemX* (housekeeping), *stx1*, *stx2* and *eaeA* genes, and a SYBR® Green real time PCR was used to monitor gene expression.

Results: Growth of the cells and *hemX* gene expression were not affected by treatment with the LMWC. The expression of *stx1* in *E. coli* O157:H7 was reduced by 74% and 72% by LMWC 1 ($P \leq 0.04$) and LMWC 9 ($P \leq 0.19$), respectively, while *stx2* gene expression was reduced by 84% and 74% by the same compounds ($P \leq 0.03$ and 0.08), respectively. The same LMWC resulted in a significant reduction of 88% and 93% ($P \leq 0.01$ and 0.03) in the expression of *stx2* in *E. coli* O111:H8, while *eaeA* gene expression was reduced by 34% and 25% ($P \leq 0.15$ and 0.23), respectively.

Significance: Results of this study show that the use of LMWC could be an alternative way to inhibit the expression of STEC virulence genes without causing cell death, which holds great potential as suitable candidates for therapeutic development.

P3-03 Impact of Market Withdrawal of Roxarsone on Arsenic Resistance in *Campylobacter* spp.

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Developing Scientist Competition

Introduction: Roxarsone, an organoarsenical, coccidiostat previously used in poultry production, is processed by microflora in the litter to harmful inorganic arsenic. This may be re-ingested by the bird causing the gut microflora to become exposed to arsenic and subsequently select for arsenic resistance.

Purpose: Given that roxarsone was voluntarily withdrawn from the market in 2011, we hypothesized that *Campylobacter* isolated from retail carcasses after 2011, will not possess the arsenic resistance genes observed in those isolated before 2011.

Methods: Thirteen *Campylobacter* isolates from retail chickens, organic and conventional, were screened for arsenic resistance. PCR primer sets specific for the arsenic resistance genes *arsC*, *acr3, arsB, arsP, and arsR* were used to determine the presence/absence of these genes in the isolates.

Results: *Campylobacter* was isolated from thirteen of thirty-four chickens purchased and sampled in 2013 (38%). Four of the isolates came from conventionally reared birds, while nine came from free-range, organically reared birds. Of the thirteen isolates, three possessed the arsenic resistance genes *arsC* and *acr3*, which regulate arsenate reduction and arsenite excretion, respectively. Two of the three isolates possessing these genes were isolated from conventionally reared birds. Only one of the thirteen isolates, a free range isolate, possessed the *arsR* gene. It was also one of the three isolates to possess *arsC* and *acr3*. Five isolates, three from organic and two from conventional, contained *arsP*, and, only one, a conventionally reared bird, also contained *arsC* and *acr3*. Five isolates possessed *arsB*, which is separate from the *ars* operon and can contribute to arsenite resistance. Of these five isolates, four were organic.

Significance: From our results, it is clear that arsenic resistance genes persist in poultry isolates of *Campylobacter* spp., even though the selection pressure has been removed. This indicates that these resistance genes do not pose a fitness burden on the organism and further research into their fitness impact should be undertaken.

P3-04 Isolation and Molecular Characterization of Multidrug-resistant *Salmonella enterica* from Imported Food Products in the U.S. during 2011 to 2013

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Introduction: One hundred nineteen *Salmonella enterica* isolates were recovered from various imported food products including fresh, frozen, or dried vegetables, fruits, meats, or seafoods during the period 2011 to 2013.

Purpose: The objective of this study was to characterize the antimicrobial resistance determinants of the *Salmonella enterica* isolates.

Methods: *Salmonella enterica* isolated from food samples were characterized for antimicrobial resistance phenotypes, genotypes, PCR detection of antimicrobial resistance genes, sequencing of integrons, plasmid analysis and transferability of antibiotic resistance by conjugation.

Results: Twenty four (20.2%) out of 119 *S. enterica* isolates were resistant to various antibiotic classes including ß-lactam, aminoglycoside, phenicol, glycopeptide, sulfonamide, or trimethoprim antimicrobials. Fourteen of the isolates were resistant to multi-antimicrobials. Four *Salmonella* isolates (PSS_903, PSS_913, PSS_984 and PSS_988) were resistant to at least 4 different classes of antibiotics used in the study. The antimicrobial resistant genes, including bla_{TEM-1}, $bla_{CTX-M-9}$, bla_{OXA-1}, *tet*(A), *tet*(B), *tet*(D), *dfrA1, dfrV, dhfrXII, drf17, aadA1, aadA2, aadA5,* and/or *orfC,* were detected. Conjugation data showed that the antimicrobial resistant genes can be transferred to *E. coli* J53. The transconjugants *E. coli* were also resistant to antibiotics like

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parent *S. enterica* strains. Plasmid profiles showed that 18 isolates (75.0%) of the 24 antibiotic resistant *Salmonella* strains harbored plasmids having incompatibility group IncFIB, IncHI1, IncI1, IncN, IncW, and IncX.

Significance: The results of this study showed that various imported food products were contaminated with *S. enterica* strains and some of them were multidrug-resistant. We report that emergence of multidrug resistance mediated by conjugative plasmids in *S. enterica* food isolates may become a global threat. Therefore, our monitoring data combined with molecular characterization of antimicrobial resistance determinants in *Salmonella* isolated from imported food products provides information that can contribute significantly to enhance food safety and public health.

P3-05 Antibiotic Resistance of Different *Staphylococcus aureus* Strains Isolated from Clinical Samples and Artisanal Cheese Produced in Costa Rica

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Introduction: In Costa Rica, there are few data regarding the antibiotic resistance profile of *S. aureus* strains isolated from cheese samples, and there is no evidence of similarity or difference from the profiles presented by clinical isolations.

Purpose: The aim of this work is to evaluate the occurrence of *S. aureus* in cheese samples, and compare the antibiotic resistance profile of these with some clinical isolates obtained from national hospitals.

Methods: *S. aureus* strains were isolated from 96 cheese samples produced in Costa Rica, using the methodology described by Pouch et al., 2011. For the determination of the resistance profile of the isolated strains, the automatized Vitek system (BioMerieux) was used. Also, 200 resistance profiles of clinical isolates were obtained from national hospitals.

Results: 172 *S. aureus* strains isolated from cheese samples and 218 clinical isolates were analyzed. None presented resistance to vancomicine or linezolid. Resistance levels were higher in the strains from clinical origin for all the antibiotics tested, except for tetracycline. Both populations presented low resistance levels to trimethoprim sulfa and nitrofurantoine.

Significance: Some *S. aureus* strains present in cheese show resistance to different antibiotics and might eventually represent an important role in the dissemination of resistance genes between bacterial groups.

P3-06 A Summary Index for Antimicrobial Resistance in Food Animals in The Netherlands

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Introduction: The Dutch government has set targets for reduction of antimicrobial usage in food animals, stipulating a 50% reduction in usage (on a weight basis) in 2013 as compared to 2009 and a 70% decrease in 2015. To evaluate the impact of this policy for public health, antimicrobial resistance (AMR) is monitored for several pathogenic and commensal bacteria (including *E. coli* and *Enterococcus* spp.) in food animals as well as foods.

Purpose: A summary index of AMR in the food chain is needed, in order to comprehensively report the impact of reducing antimicrobial usage in food animals on AMR in food-related isolates to parliament.

Methods: Scientists and policy makers contributed to the definition of a set of criteria to evaluate existing monitoring systems and relevant antimicrobial agents. Data on AMR in selected isolates from four animal species (broiler chickens, pigs, veal calves and dairy cattle) were obtained from 2009-2012. Different methods of aggregating these data were explored.

Results: Monitoring for resistance in commensal *Escherichia coli* best met the required characteristics. Antimicrobial classes selected for inclusion in the summary index were therapeutically important substances (3^{rd}/4^{th}generation cephalosporins and quinolones) and high usage substances (tetracyclines and penicillins). Resistance to critically important antimicrobials was less prevalent than to high usage antimicrobials. The mean trend anchored to the average resistance level in 2009 was preferred by policy makers.

Significance: Reducing AMR in food animals is a key policy objective in many countries around the world. This study shows that active monitoring of resistance among commensal bacteria can inform about the impact of such policies, and that detailed data can be aggregated to summary indices that are meaningful for policy makers.

P3-07 VirR: A *Listeria monocytogenes* Two-component Response Regulator Important for Resistance against Commercial Food Antimicrobial Compounds

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Introduction: The application of antimicrobial compounds during processing of ready-to-eat (RTE) foods constitutes an important safety hurdle against foodborne pathogens such as *Listeria monocytogenes*. While the efficacy of many antimicrobial compounds (e.g., nisin; NIS, ε-polylysine; EPL, chitosan; CHI) have been demonstrated in a variety of foods, the current understanding of the resistance mechanisms employed by *L. monocytogenes* to counteract these stresses is limited.

Purpose: The specific purpose of the study was to identify *L. monocytogenes* two-component system response regulators that modulate resistance to food antimicrobial compounds.

Methods: *L. monocytogenes* two-component system response regulators (i.e., liaR, lisR, virR, cesR) that have previously been determined to have a role in responding to cell envelope stress were deleted via allelic exchange method and screened for antimicrobial sensitivity using a minimum inhibitory concentration (MIC) assay in brain heart infusion (BHI) broth. In addition, we assessed the viability of the wild type (WT) and response regulator mutants following exposure to the antimicrobial stress using the Live/Dead staining procedure.

Results: The data from the MIC assay indicated that DvirR was 16-, 4-, and 4-fold more sensitive to NIS, EPL, and CHI, respectively, compared to the WT. A significantly higher proportion of 'dead' populations as indicated by the nucleic acid stain ratio (SYTO-9 to propidium iodide) was observed for DvirR compared to the WT under NIS, EPL, and CHI stress ($P < 0.05$), indicating that VirR contributes to overcoming the antimicrobial stress.

Significance: Identifying regulators involved in antimicrobial stress response is critical for elucidating potential resistance mechanisms. The mechanistic understanding of the resistance mechanisms can then foster opportunities for further improvement in food antimicrobial intervention strategies as these regulatory feature(s) can be exploited using combination treatments that have knowledge-based rationale.

P3-08 Validation of a Less-Than-Daily Sanitation Program for a Poultry Water Chilling System

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Introduction: Poultry companies are investigating the option of implementing "Less-than-daily" sanitation programs to reduce downtime needed for sanitation.

Purpose: To validate a water recycling system supplied with peroxyoctanoic acid (POOA, 30-40ppm) that will allow the plant to hold over the water used in a poultry chiller for multiple days.

Methods: Trials were conducted to assess the microbiological effects on turkey carcasses, water chiller media, and chiller surfaces during a 35 day baseline period of normal sanitation practices and a 68 day period of "Less-than-daily" (LTD) sanitation practices in which the media in the chiller was held over for periods of 5 days. *E. coli*, *Enterobacter* and aerobic plate counts (APC) populations were recovered from carcass samples. *E. coli* and APC populations were collected from water and contact surface samples. The water chiller media was treated with POOA during baseline and LTD periods. During the LTD period a water recycling system was utilized. Data was analyzed using Minitab Two-Sample *t*-test.

Results: There were significant ($P < 0.05$) reductions in *E. coli* and APC populations recovered from the carcass samples from LTD period vs. baseline period. LTD *E. coli* and APC results were 0.163 ± 0.44 and 1.24 ± 0.87 CFU/ml, respectively, vs. baseline results of 0.39 ± 0.64 and 1.81 ± 0.74 log CFU/ml, respectively. There were also significant ($P < 0.05$) reductions in APC populations recovered from contact surface and water samples. LTD contact surface APC results were 1.08 ± 1.02 vs. baseline results of 1.50 ± 1.00 log CFU/ml and LTD water APC results were 0.56 ± 0.78 vs. 0.90 ± 0.91 log CFU/ml for baseline. Although, not statistically significant ($P > 0.05$), all other LTD test results were lower than or equal to baseline results.

Significance: These data indicate that water in a poultry chiller supplied with POOA and a recycling system can be held over for multiple days with no impact on the carcasses, contact surfaces or water quality.

P3-09 Use of a Nucleic Acid Aptamer-based Method to Study Thermal Inactivation of Human Norovirus

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Developing Scientist Competition

Introduction: The inability to cultivate human noroviruses (hNoV) *in vitro* prevents accurate characterization of the efficacy of inactivation methods. Because viral RNA persists long after loss of infectivity, reverse transcription (RT)-qPCR detection methods are not able to determine virus infectivity status. Capsid integrity and functionality based on binding to virus-specific ligands (antibodies, histo-blood group antigens) has been used to approximate hNoV infectivity in inactivation studies; however, results have been mixed.

Purpose: To use an alternative binding ligand (nucleic acid aptamer) to aid in discrimination of hNoV infectivity status as a function of heat treatment.

Methods: Virus-like particles (VLPs) of hNoV GII.2 were exposed to temperatures from 65°C to 80°C for various times. Capsid integrity was assessed based on binding ability to previously characterized ssDNA aptamers using an Enzyme-Linked Aptamer Sorbent Assay (ELASA). Transmission electron microscopy images of the treated capsids were produced for comparison.

Results: Treatment of VLPs at 70°C for 20 and 40 minutes produced reductions in ELASA signal intensities of 33.9 ± 4.9% and 71.9 ± 0.5%, respectively. At 72°C, signal intensity reductions of 59.6 ± 2.5% and 74.0 ± 0.3% were observed after 10 and 20 minutes of heat, respectively. Treatments of 75°C and 80°C for one minute resulted in 45.1 ± 4.8% and 81.8 ± 6.1% loss of ELASA signal intensity. Signal intensities at different selected temperatures for most selected time points were statistically significantly different ($P < 0.01$). These results were supported by TEM, which showed VLP morphology alteration at similar temperatures.

Significance: Reduction in ELASA signal intensity corresponded with increased time-temperature exposures of hNoV VLPs, suggesting that aptamer binding may be a cheaper, readily-accessible alternative to other virus-specific ligands (antibodies, histo-blood group antigens) used for discriminating hNoV infectivity. Studies are underway to determine z_D values using aptamer binding in conjunction with RT-qPCR.

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P3-10 Thermal Inactivation of Shiga Toxin-producing *Escherichia coli* (STEC) in Seasoned Ground Beef Products Supplemented with Clean Label Antimicrobial Ingredients

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Introduction: USDA recommends cooking ground beef to 71.1°C to kill pathogens such as Shiga toxin-producing *E. coli* (STEC). However, undercooking by consumers or in food service may result in increased risk of foodborne illness.

Purpose: This study was designed to determine if the addition of clean-label antimicrobials enhance the thermal inactivation of STEC in seasoned ground beef cooked at 60°C (140°F).

Methods: Thermal inactivation of STEC in a Control seasoned ground beef product was compared to treatments supplemented with 0.5% or 1.0% smoke flavoring fraction (SF; Zesti AM 10) or 0.8% dried vinegar (DF; DuraFresh™ 2016). Products were homogenized with either a 3-strain mixture of *E. coli* O157:H7 or a 6-strain mixture of non-O157 STEC (including O26, O45, O103, O111, O121, O145) to yield 8-log CFU/g. Samples (1 g portions) were flattened into thin films in moisture-impermeable pouches, vacuum-sealed, and heated to 60°C in a water bath. Triplicate samples were removed at 0, 2.5, 5, 7.5 and 10 minutes and immediately chilled to < 4°C. STEC was enumerated by plating on Sorbitol MacConkey agar overlaid with nonselective Trypticase Soy agar to enhance recovery of injured cells. Study was replicated twice.

Results: D-values at 60°C for the Control were 2.03 and 2.19 min for O157 and non-O157 STEC, respectively; D-values for products supplemented with 0.5% SF were not significantly different ($P > 0.05$). In contrast, addition of 1% SF reduced D-values to 1.45 and 1.34 min for the O157 and non-O157 treatments, respectively ($P < 0.05$). Although the additional of 0.8% DF reduced D-values of O157 to 1.02 min ($P < 0.05$), it did not enhance the thermal inactivation of non-O157 STEC compared to the control (D_{60}=1.75 min; $P > 0.05$).

Significance: This study demonstrated that addition of certain clean-label antimicrobials can safely reduce the cook time for seasoned ground beef by enhancing the thermal inactivation of STEC, but STEC serotypes differ in sensitivity.

P3-11 Inhibitory Activity of Hydrogen Peroxide, Water and Organic Sanitizers against *Escherichia coli* O157:H7 on Organic Leafy Greens

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Introduction: Increased consumption of fresh produce in the past decade has been accompanied with an increase in related foodborne outbreaks. *Escherichia coli* O157:H7 has often been the cause of these occurrences. Organic leafy greens have also been implicated in foodborne outbreaks in recent years. However, due to the limited number of approved sanitizers for organic produce, it is necessary to derive and test new alternatives.

CHICO wash is an organic sanitizer that has been shown to reduce *E. coli*, *Salmonella*, and *Clostridium botulinum* contamination by 7 logs. However, very few sanitizers with an organic make-up have been tested against foodborne pathogens in organic leafy greens.

Purpose: The antimicrobial efficacies of three different organic sanitizers were evaluated on organic leafy greens contaminated with *E. coli* O157:H7.

Methods: Three organic formulations at various concentrations: C8.C10 and CG100 (at .2 and .4%) and CHICO ($C_3H_8I_0C_3O_7$ Wash; 5%), along with the controls: positive, hydrogen-peroxide and water, were tested. Organic baby and adult spinach and romaine and iceberg lettuce were inoculated with a cocktail of *E. coli* O157:H7 strains at 10^6 CFU/ml. The contaminated greens were washed in each treatment for 1 or 2 minutes, followed by a wash in D/E neutralizing broth. Greens were then bagged and stored at 4°C. Surviving bacterial populations documented on days 0, 1, and 3 of storage.

Results: With the CHICO treatment, both wash times displayed significant reduction ($P < 0.05$) of *E. coli* O157:H7 population. Compared to the positive control, CHICO and hydrogen-peroxide preformed similarly. Compared to water, CHICO wash reduced the pathogen population by more than 3 logs on Day 0. Both hydrogen-peroxide and CHICO reduced *E. coli* O157:H7 populations linearly across the sampling period.

Significance: This study demonstrates the potential antimicrobial effect of organic sanitizers against *Escherichia coli* O157:H7 strains on organic leafy greens.

P3-12 Investigating the Responses of *Cronobacter sakazakii* to Garlic-derived Organosulfur Compounds: A Systematic Study of Pathogenic-bacterium Injury by Use of High-throughput Whole-transcriptome Sequencing and Confocal Micro-raman Spectro

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Introduction: *Cronobacter sakazakii* is a leading pathogen associated with invasive infection of infants and causing meningitis, necrotizing enterocolitis, and bacteremia.

Purpose: Garlic-derived organosulfur compounds are found to be highly effective antimicrobial compounds against different foodborne pathogens, resulting in less antimicrobial resistance.

Methods: We used high-throughput whole-transcriptome sequencing (RNA-seq) and vibrational spectroscopy to characterize and fingerprint *C. sakazakii* injury under treatment of two garlic -derived organosulfur compounds (diallyl sulfide and ajoene).

Results: Both diallyl sulfide and ajoene are highly effective in inactivating *C. sakazakii*. RNA-seq analyses showed that the bacterial response to ajoene differed from the response to diallyl sulfide. Specifically, ajoene caused downregulation of motility-related genes, while diallyl sulfide treatment caused an increased expression of cell wall synthesis genes. Confocal micro-Raman spectroscopy revealed that the two compounds appear to have the same phase I antimicrobial mechanism of binding to thiol-containing proteins/enzymes in bacterial cells generating a disulfide stretching band but different phase II antimicrobial mechanisms, showing alterations in the secondary structures of proteins in two different ways. Diallyl sulfide primarily altered the α-helix and β/-sheet, as reflected in changes in amide I, while ajoene altered the structures containing phenylalanine and tyrosine. Scanning electron microscopy confirmed cell injury, showing significant morphological variations in cells following treatments by these two compounds.

Significance: Findings from this study aid in the development of effective intervention strategies to reduce the risk of *C. sakazakii* contamination in the food production environment and on food contact surfaces, reducing the risks to susceptible consumers.

P3-13 An Alternative Antimicrobial Commercial Egg Washing Procedure

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Developing Scientist Competition

Introduction: Commercial eggs are washed with an alkaline detergent at approximately pH 11 followed by a chlorine rinse. At this pH, little, if any, free chlorine is in the final rinse to act as an antimicrobial against pathogens like *Salmonella*. Using a chlorine stabilizer (e.g., Smartwash) in the wash may help maintain chlorine effectiveness.

Purpose: The objective was to determine the most effective egg wash treatment. A wash treatment using SmartWash and chlorine at a pH of 6 and the standard alkaline wash treatment, both followed by a chlorine rinse, were compared. The effect of wash water temperature (ambient or 49°C (120°F)) for both treatments was also compared. Treatments were evaluated based on antimicrobial effectiveness and effect on egg quality.

Methods: Eggs were washed using both wash solutions at ambient temperature or 49°C (120°F) and evaluated for various egg quality parameters (shell strength, shell stiffness, shell color, Haugh Unit, vitelline membrane strength, and total solids) biweekly over 12 weeks. In the second phase, eggs were inoculated with *Salmonella* Typhimurium and washed using the 4 treatments. *Salmonella* levels before and after washing were compared to determine intervention effectiveness.

Results: Compared to the alkaline wash, SmartWash treated eggs had higher shell strength, Haugh Unit, vitelline membrane strength, and b* measurements. Eggs washed at ambient temperatures had greater shell stiffness. No differences in total solids were noted. For both temperature treatments, *Salmonella* levels on eggs treated with SmartWash were reduced by >2.5 logs compared to reductions of <2.3 logs on alkaline treated eggs. Total aerobic counts were reduced by >2.6 logs on SmartWash treated eggs compared to 2.0 logs on alkaline treated eggs.

Significance: A chlorine stabilizer like SmartWash could be used as an effective antimicrobial for table eggs without compromising egg quality. There are potential energy savings using ambient wash temperatures.

P3-14 Quantification of *Carnobacterium maltaromaticum* Bacteriocin Gene Expression on Refrigerated Vacuum-packaged Ham

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Developing Scientist Competition

Introduction: *Carnobacterium maltaromaticum* is a bacteriocin-producing protective culture approved for use in food. Bacteriocins produced by *C. maltaromaticum* ATCC®PTA-5313 inhibit the growth of *Listeria monocytogenes;* when used in combination with other antimicrobials, *L. monocytogenes* on ready-to-eat meats is killed to levels below the detection limit. *C. maltaromaticum* produces three different bacteriocins, carnocyclin, piscicolin 126 and carnobacteriocin BM1; however, it remains unclear which of the three compounds is responsible for the preservative effect on meat.

Purpose: The aim of this study was to quantify the gene expression of the three bacteriocins produced by *C. maltaromaticum* ATCC®PTA-5313 on vacuum packaged ham stored at 4°C.

Methods: Ham was inoculated with 10^3 CFU/cm^2 *C. maltaromaticum* ATCC® PTA-5313, vacuum packaged and stored at 4°C for up to 28 days. Samples were taken throughout storage for quantification of cell counts, and for RNA isolation. RNA was extracted using the Qiagen RNeasy kit and followed by reverse transcription. Quantitative Real Time Polymerase Chain Reaction was used to determine expression of the structural genes for carnocyclin A (*cclA*), piscicolin 126 (*pisA*), carnobacteriocin BM1 (*cbnBM1*) and housekeeping gene *16SrRNA*. Data from three replicates was analyzed using the comparative C_T method.

Results: The expression of *cclA, pisA,* and *cbnBM1* was quantified from refrigerated ham. Cell counts indicated that *C. maltaromaticum* ATCC® PTA-5313 was present at levels between 6-8 log CFU/cm^2 when RNA was extracted. C_T values lower than 35 were considered positive for expression of the bacteriocins. $C_T$values on day 21 and 28 for carnocyclin A were between 21.24 to 24.44, piscicolin 126 ranged from 22.8 to 31.6 and carnobacteriocin BM1 ranged 23.4 to 27.1.

Significance: This method could be used to confirm the production of bacteriocins by *C. maltaromaticum* ATCC® PTA-5313 on ready-to-eat meats to control the growth of *L. monocytogenes*.

P3-15 Evaluation of Multiple Temperatures of Lactic Acid and Sodium Metasilicate on Microbial Parameters of Fresh Beef

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Introduction: Lactic acid and sodium metasilicate have been used in meat facilities as antimicrobial interventions. Their uses have varied from hot carcasses to ready-to-eat products. Utilizing these antimicrobials in different stages during meat processing may assist in the reduction of the risk of pathogenic microorganisms.

Purpose: The purpose of this study was to determine optimum temperatures of usage of lactic acid and sodium metasilicate for pathogen reduction on beef bottom round muscles.

Methods: Lactic acid 4% (LA, v/v), sodium metasilicate 4% (SM, w/v), the combination of the two solutions (LASM), and a distilled water control were applied at 4, 25, and 60°C. Antimicrobials were mixed in solution with distilled water. Beef bottom round was cut into 100 cm^2 pieces. Pieces were inoculated with *Escherichia coli* O157:H7 (5 strains), Non-O157 Shiga toxin-producing *Escherichia coli* (STEC, 1 strain each of the "Big 6"), *Salmonella* spp. (5 strains), or *Listeria monocytogenes* (5 strains). After 30 min of contact time samples were treated with the antimicrobial solution or control and then allowed 30 min of contact time. Samples were serially diluted and plated on MacConkey Agar with Sorbitol (*E. coli*), XLT4 (*Salmonella* spp.), or Modified Oxford Medium (*L. monocytogenes*). Data were analyzed using the PROC MIXED procedure of SAS and Tukey pairwise comparisons.

Results: LA and SM reduced ($P < 0.05$) the microbial contamination of the meat samples. Temperature of application had no effect ($P < 0.05$) on bacterial counts in any of the treatments. LA or SM alone were more effective ($P < 0.05$) in reduction of microbes than when used together (LASM).

Significance: Meat processors can apply LA or SM at refrigeration temperatures and reap the same benefits of applying them at a higher temperature. Both solutions can serve as a hurdle technology in meat processing facilities.

P3-16 Lactic Acid Bacteria as a Biocontrol Agent to Inhibit *Listeria monocytogenes* during Sprouting of Alfalfa Sprouts

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Introduction: Very few biological interventions using competitive exclusion techniques have been validated for effectiveness against *L. monocytogenes* on alfalfa sprouts.

Purpose: The objective of this study was to evaluate the synergistic effect of the lactic acid bacteria (LAB) strains, NP7 (*Lactobacillus lactis*) and FS56 (*Lactococcus lactis*), on the growth and survival of *L. monocytogenes* (strains: Brie, Scott A, Murray B and Auburn 311) on sprouted alfalfa seeds.

Methods: Four LAB treatments [(1) NP7 seed soak and NP7 sprout spray, (2) FS56 seed soak and FS56 sprout spray, (3) FS56 seed soak and NP7 sprout spray, (4) NP7/FS56 seed soak and NP7/FS56 sprout spray] were applied at 8.00 log CFU/ml to alfalfa sprouts containing pathogen concentration of approximately less than 1.00 log CFU/g. Two identical Sprouting apparatus were run simultaneously so that each trial with co-culture inoculated seeds (*L. monocytogenes* and LAB) could be accompanied with a matched control (*L. monocytogenes* only) over the course of five days. *L. monocytogenes* and LAB were enumerated everyday on Modified Oxford agar (MOX) and De man, Ragosa, Sharpe agar (MRS), respectively.

Results: In comparison with the control sample, sprouts treated with only a single LAB strain (Treatments 1 and 2) had significant reduction of *L. monocytogenes* ($P < 0.05$) on days 1 and day 2 of sprouting. In the sprouts treated with a co-culture LAB (treatments 3 and 4), there was significant reduction ($P < 0.05$) on all days. The most significant reductions were observed for treatment 4, (4.41 log CFU/g) reduction and no detection of pathogen cells by direct plating at day 5.

Significance: The co-culture LAB treatments displayed stronger inhibition activity than the single LAB strain treatments, and this method might prove to be an efficacious natural method to control pathogen outgrowth during the sprouting stages of alfalfa sprouts.

P3-17 Cytotoxicity of Bacteriocins Produced by Four *Enterococcus faecium* Strains Isolated from Cheese

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Introduction: Bacteriocins are compounds produced by several bacterial strains in order to compete with other microorganisms contained in the same micro-habitats. In recent years, these compounds have been proposed for use as biological preservatives for certain food products. Bacteriocin-producing bacteria are relatively common but often these substances may have in addition of bactericide effect, a profile of toxicity to humans and animals that impedes the use of these substances.

Purpose: Therefore, the aim of this study was to analyze the cytotoxicity of partially purified bacteriocins isolated from four different bacterial strains.

Methods: Bacteriocins produced by *Enterococcus faecium* ST209GB, ST278GB, ST315GB and ST711GB were partially purified from 24-hour cultures grown in MRS broth by precipitation with ammonium persulfate and hydrophobic chromatography separation column SepPakC18. Huh7.5 cells derived from human hepatocarcinoma were seeded in 96-well plate and subsequently treated with increasing concentrations of bacteriocins. Two days after initial treatment, MTT was added to cells and colorimetric intensity was obtained at wavelength of 562 nm. The values obtained were transformed into percentage of viability compared to negative control (untreated).

Results: The tested bacteriocin concentrations ranged from 10 µg/ml to 160 µg/ml. In two strains (ST315GB and ST711GB) of four tested bacteriocin the CC_{50} value was close to 80 µg/ml. Moreover, for the substance produced by the strain ST209GB CC_{50} was approximately of 160 µg/ml. Finally, the viability of the bacteriocin produced by the strain ST278GB, at the highest tested concentration was 85%, and therefore it was not possible to estimate the value of CC_{50}. All strains showed cell viability above 90% when tested at a concentration of 10 µg/ml.

Significance: In several studies attention has focused on the antimicrobial properties of bacteriocins, however, as evidenced in this work, often these compounds have a cytotoxicity profile that would impede the use of these substances as biopreservatives.

P3-18 Studies of the Effect of Two Bacteriocinogenic Starter Cultures on Growth of *Listeria innocua* and Sensory Properties of a Traditional Cured/smoked Sausage-like Product

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Introduction: Traditional cured/smoked sausage-like products are much appreciated by south western European consumers. However, modern consumers require products that not only have high appealing sensory attributes but are also safe.

Purpose: The purpose of this study was to ascertain the effect of the inoculation of two bacteriocinogenic cultures on growth of *L. innocua* and on organoleptic characteristics of "Alheira", a traditional cured/smoked sausage-like product.

Methods: *Lactobacillus sakei* ST153 and BLC35 (commercial starter culture including strains of *Lactobacillus curvatus*, *Staphylococcus xylosus* and *Pediococcus acidilactici*; CHR Hansen) were used as bacteriocinogenic cultures. Six batches of "Alheiras" were manufactured: ST153 (inoculated with *Lact. sakei* ST153), BCL-35 (inoculated with BCL-35), ST153+L (inoculated with *Lact. sakei* ST153 and *Listeria innocua*), BCL-35+L (inoculated with BCL-35 and *L. innocua*), C (control batch without added strains) and C+L (control batch inoculated with *L. innocua*). Each trial was performed in triplicate. For sensory analysis three batches of "Alheira" were tested: ST153, BLC-35 and C. A quantitative descriptive sensory test, using 12 descriptors, was performed by a sensory trained panel at four sessions. "Alheiras" were packed under MAP (20 % CO_2 and 80% N_2v/v) and stored at 4°C during 66 days.

Results: A significant reduction in the levels of *L. innocua* in samples inoculated with bacteriocinogenic cultures was found along storage. *L. innocua* decreased ca. 2 log CFU g^{-1} during the first 12 hours of storage. With respect to visual and texture descriptors the sensory panel didn't detect differences between the samples compared to the control, but they have detected differences (P < 5%) in flavour descriptors, namely, low characteristic taste, high acid taste and low conformity during shelf life.

Significance: These data suggest that the bacteriocinogenic cultures tested can be used as a means to improve the microbiological safety of "Alheira".

P3-19 Antimicrobial Effect of Carvacrol and Cinnamaldehyde against *Salmonella* Tennessee in a Low Water Activity Model

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Developing Scientist Competition

Introduction: Salmonellosis caused by consuming low water activity (a_w) foods contaminated by *Salmonella* has been frequently reported. However, methods to control *Salmonella* in these foods, especially chemical control methods, have not been widely explored.

Purpose: To evaluate the antimicrobial activity of carvacrol and cinnamaldehyde against *Salmonella* Tennessee in a low water activity model over 72h at 25°C.

Methods: Model solutions at a_w 1.0, 0.7, 0.5 and 0.3 were made using glycerol and sterile deionized H_2O, with 5% (w/v) sucrose. Carvacrol and cinnamaldehyde stocks were made in 95% ethanol and added to each a_w solution either individually to achieve final concentrations of 0, 125, 250 and 500 ppm or together with each compound at 125 or 250 ppm. All solutions were inoculated with stationary phase *S.* Tennessee K4643 and incubated up to 72h at 25°C. Samples were taken every 24h, serially diluted, and plated on tryptic soy agar. Each experiment was performed in triplicate.

Results: Reduced a_w (0.7, 0.5 and 0.3) alone decreased the viable population over time with greater reduction at lower a_w. With antimicrobials, greater inactivation was seen at higher a_w. At a_w 1.0 and 0.7, a >7 log CFU/ml reduction in population was observed within 24h with 500 ppm carvacrol. Cinnamaldehyde was more effective than carvacrol at a_w 0.5 and 0.3 (2.7-2.9 vs. 0.39-1.97 log reduction). When combined, possible synergistic effects of the two were observed at a_w 1.0 and 0.7 but not at lower a_w.

Significance: Both essential oil components have the potential to be used in reduced a_w foods. Cinnamaldehyde was more effective than carvacrol at a_w < 0.5. The combination of the two caused greater inhibition than would have been expected with the compounds used alone at higher a_w but possible antagonistic effects were observed at lower a_w.

P3-20 The Efficacy of Chitosan Coating in Reducing Surface Attached *Salmonella* on Tomatoes

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Introduction: A microbial safety concern with tomatoes is related to several *Salmonella* outbreaks. Alternative and novel methods are needed to enhance the microbial safety of tomatoes following a standard chlorine wash step. Chitosan is an edible, biodegradable, non-toxic and antimicrobial active compound.

Purpose: In this study, the efficacy of chitosan to control *Salmonella*, yeast and mold, and commensal bacteria associated with tomatoes was evaluated.

Methods: Tomatoes (n = 172) were inoculated with *Salmonella* to achieve 10^4 CFU/g. All tomatoes were dipped in 100 ppm chlorine water for 90 seconds. After drying, tomatoes were dipped in 0.2% chitosan solution for 90 seconds. Controls were not treated with chitosan. Post-treatment tomatoes were stored at 15°C and 80-90% humidity for 15 days. The population of commensal bacteria, *Salmonella*, yeast and mold were evaluated at pre-determined times.

Results: The initial population of commensal bacteria, *Salmonella*, yeast and mold were 5.65, 4.44 and 4.05 log CFU/g, respectively. Immediately post-treatment, a 4.02-log CFU/g reduction in commensal bacteria was achieved on treated compared to a 3.33-log CFU/g reduction on control tomatoes. Up to a 4-log CFU/g reduction in *Salmonella* was achieved compared to 3.25-log CFU/g reduction in control. Yeast and mold populations immediately post-treatment decreased by 2.43 log CFU/g, compared to a 1.93-log CFU/g reduction in control. By 24h post-treatment the population of

commensal bacteria, *Salmonella,* yeast and mold increased to pre-treatment levels on both control and treated tomatoes and then remained constant through 15 days of storage.

Significance: Treatment of tomatoes with 0.2% chitosan effectively reduced populations of *Salmonella,* yeast and mold. These results serve as the base for development of a product that improves product safety and shelf life.

P3-21 Biological Control of *Salmonella* Biofilm on Stainless Steel Surfaces

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Developing Scientist Competition

Introduction: *Salmonella* spp. may be introduced into a rendering processing plant receiving area through raw materials and may persist by forming biofilms on surfaces.

Purpose: This study was developed to study the efficacy of bacteriophages for reducing *Salmonella* biofilms on surfaces and ultimately for preventing *Salmonella* re-contamination of rendered animal by-products and animal feed.

Methods: *Salmonella* isolates obtained from rendering facilities were characterized using pulsed field gel electrophoresis (PFGE) and serotyping analysis. Seven bacteriophages were selected for phage treatment based on evaluating host ranges of 94 bacteriophages against selected *Salmonella* isolates. Under laboratory conditions, the effectiveness of phage treatment with a multiplicity of infection (MOI) of 100 was evaluated against strong *Salmonella* biofilm formers using a colorimetric method in 96-well microplate. A greenhouse study was conducted to simulate real-world conditions in a rendering plant and phage treatment with MOI of 1,000 was applied for 7 days to reduce *Salmonella* biofilm formed on stainless steel surfaces in different seasons.

Results: A total of 34 samples (63%) were *Salmonella*-positive after enrichment and 166 *Salmonella* isolates were obtained sharing 31 different PFGE patterns and 16 different serotypes. The reduction of *Salmonella* biofilm reached up to 90% with phage treatment under laboratory conditions. In the greenhouse study, phage treatment reduced up to 3.4 log (99.96%) and 1.4 log (96%) CFU/cm^2 of biofilm formed by *Salmonella* in summer (Ave. 28ºC; Ave. relative humidity: 67%) and fall/winter (Ave. 21ºC; Ave. relative humidity: 32%) seasons, respectively.

Significance: The results of this study demonstrated the effectiveness of phage treatment in reducing *Salmonella* biofilm on the surface of stainless steel under laboratory and greenhouse conditions. This suggests use of bacteriophages in rendering facilities that utilize stainless steel feed contact surfaces may have merit in reducing the likelihood of finished rendered products being re-contaminated with *Salmonella*.

P3-22 Isolation and Characterization of Bacteriophages of *Listeria monocytogenes* with Potential to be Used as Biocontrol Agents for Food Safety

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Poster

Introduction: Since the foodborne pathogen *Listeria monocytogenes* is common in dairy farm environments, it is likely that bacteriophages infecting this bacterium are abundant on raw milk samples. Significant progress has been made toward their applications in food and has resulted in the U.S. Food and Drug Administration approving the use of a bacteriophage-based additive for the control of *Listeria monocytogenes* in foods.

Purpose: To better understand the diversity and host range of listeriaphages on raw milk samples and to develop a diverse phage collection for further studies.

Methods: A total of 10 raw milk samples were collected from a dairy farm in Sao Paulo state between July and December 2013. Six bacteriophages of *L. monocytogenes* were isolated using an enrichment protocol and the double agar layer method. The host ranges of the six bacteriophages were determined by performing spot tests with nine *L. monocytogenes* strains, including serotypes 4b, 4a, 1/2a, 1/2b.

Results: While none of raw milk samples tested positive for *L. monocytogenes*, 30% of samples were positive for listeriaphages, containing up to > 2.5x10^2 PFU/ml. Host range characterization of the six phage isolates obtained, with a reference set of nine *L. monocytogenes* strains representing the major serotypes and four lineages, revealed considerable host range diversity. Serotype 1/2a strains were highly susceptible to phages and were lysed by 60 to 85% of phages tested. Overall, 34% of phage isolates showed a narrow host range (lysing 1 to 4 strains), while 67% of phages represented broad host range (lysing > 7 strains).

Significance: The extensive host range of phages observed suggests an important role of phages in the ecology of *L. monocytogenes* on dairy farms. In addition, the phage collection developed here has the potential to facilitate further development of phage-based biocontrol strategies (e.g., in cheese and milk) and other phage-based tools.

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P3-23 Evaluation of the Effects of a Newly Designed Antimicrobial Solution on *Listeria monocytogenes* and Ready-to-Eat Meat Shelf-life Quality

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Introduction: The lack of heating gives ready-to-eat (RTE) products risks for *Listeria monocytogenes* contamination. To ensure the safety of RTE products, antimicrobials are usually added during meat processing. However, the need to develop solutions that can be used at deli counters (where the deli meat can get contaminated or cross-contaminated) to spray on meat and to further ensure safety still exists.

Purpose: The objective of this study is to evaluate the antimicrobial effects of a newly designed solution made with generally recognized as safe (GRAS) ingredients and its application in RTE meats.

Methods: This antimicrobial solution is made of lauric arginate ester, nisin, and chitosan. A 3-stage study was carried out using a 5-strain *Listeria* cocktail with 2 inoculation levels: stage 1 was designed to evaluate the effectiveness of this solution (2 different concentrations) on *Listeria* proliferation; stage 2 was designed to study the performance of this solution at different meat storage times; stage 3 was conducted to find out the impact of this solution on meat quality. At each stage, two separate trials were conducted and the statistical analysis was done using SAS®.

Results: Without any antimicrobials, the numbers of *Listeria* inoculated onto the roast beef increased (up to ~6 log CFU, $P < 0.05$), while on the other hand, both the 1:5 dilution and the original stock antimicrobial solutions inhibited the growth of *Listeria* significantly ($P < 0.05$). The antimicrobial effects of the solution were influenced by the meat shelf life, but no significant change was caused by this solution on meat quality.

Significance: This newly designed GRAS antimicrobial solution can inhibit the growth of *Listeria* significantly and does not cause any changes to meat products. It is a solution with good potential to be used at deli counters to further reduce and control *Listeria* contamination and proliferation.

P3-24 Antilisterial Activity of Lactic Acid Bacteria as Influenced by Temperature, Incubation Period and Culture Media

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Introduction: *Listeria monocytogenes* are regarded as a major safety problem because they can cause serious illness and death. Lactic acid bacteria (LAB) have been found to be effective in inhibiting or killing *L. monocytogenes* because of their antimicrobial metabolites.

Purpose: The objective of this study was to determine the influence of temperature, incubation period and culture medium on the antilisterial activity of LAB.

Methods: Three culture media (BHI, MRS and TSB broth) were inoculated with a *L. monocytogenes* cocktail at 10^5 CFU/ml. Then 7 LAB strains previously selected (NP3, NP35, NP51, FS56, FS92, FS97 and RP1) were individually inoculated at a level of 10^8 CFU/ml. The cultures were held anaerobically at 37°C for 72 hrs and at 5°C for 7 days, respectively. The viable counts of *L. monocytogenes* and LAB, as well as pH changes of media over time were measured.

Results: At 37°C, the highest *L. monocytogenes* reduction (9.2 log CFU/ml) was obtained by FS97 in BHI broth at hr 72, at 5 °C, FS56 led to the highest reduction (5.3 log CFU/ml) in BHI broth on day 7, and the lowest media pH (3.9) was obtained with NP35 in MRS broth at 37°C. In most cases, there were not significant ($P > 0.05$) changes in pH at 5°C compared to the control. The viable count changes of 7 LAB strains in three media under different temperatures were strain-specific for the bacteria.

Significance: Results from this study showed that LAB inhibited the growth of *L. monocytogenes* involving several factors and these factors played different role according to the growth conditions, and it seems that the mechanisms of these factors are not well understood and the consequences are not easily predictable. However these factors are able to significantly influence the microbiological safety and quality of food.

P3-25 Efficacy of Sorbate and Benzoate in Controlling the Growth of *Listeria monocytogenes* on Meat Surface

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Introduction: Treating the surface of meat products with antimicrobials to control surface-contaminated *L. monocytogenes* is an alternative measure to adding the antimicrobials into meat products. While many organic acids are frequently used for surface treatment of meat, sorbate and benzoate are GRAS antimicrobials primarily used in foods and beverages as antifungal agents.

Purpose: This study examined the efficacy of sorbate and benzoate at acidic pH in controlling *L. monocytogenes* on the surface of cooked meat.

Methods: A 5-strain mixture of *L. monocytogenes* was inoculated onto cooked ham slices to a level of approximately 3.0 log CFU/g. Inoculated samples were exposed to 0% (control), 2%, and 4% potassium sorbate or benzoate solutions adjusted with lactic acid to pH 4, 5, and 6 for 30 min, vacuum-packaged, and stored at 4°C for up 56 days.

Results: The lag phase durations (LPDs) of *L. monocytogenes* in control samples treated with pH 4, 5, and 6 solutions were <4 days and the growth rates (GRs) were 0.11, 0.18 and 0.19 log/day, respectively. *L. monocytogenes* was not able to grow in samples treated with 2% or 4% sorbate solution of pH 4, whereas the LPDs were >6 days and the GRs were 0.11 and 0.04 log/day in samples treated with 2% and 4% sorbate solutions of pH 5, respectively. Generally, sorbate was more effective than benzoate in controlling the growth of *L. monocytogenes* on cooked ham. Treatments with sorbate solutions of pH 4 and 5 were more effective in extending LPDs and reducing GRs of *L. monocytogenes* than acid solutions, whereas benzoate was more effective at pH 4.

Significance: Results suggest a potential use of sorbate or benzoate solutions at acidic pH as a surface treatment to control the growth of contaminated *L. monocytogenes* on meat surface, thereby enhancing the microbiological safety of meat products.

P3-26 *Listeria monocytogenes* and *Staphylococcus aureus* Inhibition by Mexican Oregano (*Lippia berlandieri* Schauer) Added to Starch Edible Films at Different pH Values

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Introduction: Essential oils (EOs) possess antibacterial properties and have been screened as potential sources of novel antimicrobial compounds. EOs could act as biopreservatives, reducing or eliminating pathogenic bacteria and increasing the overall quality of animal and vegetable food products. In this sense, edible films can incorporate these extracts to provide microbiological stability and reduce the risk of microbial growth on food surfaces.

Purpose: The aim of this study was to evaluate the inhibitory effect of starch edible films added with Mexican oregano (*Lippia berlandieri* Schauer) EO at different pH values on *Listeria monocytogenes* and *Staphylococcus aureus*.

Methods: Starch edible films were formulated with Mexican oregano EO concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, or 2.00%, each concentration at pH values of 5, 6, or 7. Minimum inhibitory concentrations (MIC) were determined by agar and broth diffusion methods.

Results: There was a significant difference ($P < 0.05$) in MIC values between the two diffusion methods. In the agar diffusion method pH values 5 and 6, with 2.00 and 4.00% of EO, inhibit *S. aureus* and *L. monocytogenes,* respectively; moreover, the broth diffusion method showed lower MIC values for *L. monocytogenes* (0.25%), regardless of pH values.

Significance: Starch edible films, with acid pH values, added with Mexican oregano essential oil, are effective to inhibit *Listeria monocytogenes* and *Staphylococcus aureus.*

P3-27 Inhibition of *Bacillus cereus* and *Staphylococcus aureus* by Coriander (*Coriandrum sativum*), Wild Epazote (*Teloxys graveolens*) and Papalo (*Porophyllum ruderale*) Extracts

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Introduction: Today the consumer demands for less use of synthetic food preservatives have increased throughout the world. In this context, plant extracts have gained considerable attention as antimicrobial and have formed the basis of many applications like food preservation. Is known that various plant extracts have activity against the Gram-positive and Gram-negative of foodborne pathogens.

Purpose: The aim of this study was to evaluate the inhibitory effect of chloroform extracts of three commonly use culinary Mexican plants, coriander (*Coriandrum sativum*), wild epazote (*Teloxys graveolens*) and papalo (*Porophyllum ruderale*) on *Bacillus cereus* and *Staphylococcus aureus*.

Methods: Plants were collected at traditional markets in Puebla, Mexico. Extracts were prepared by extracting plant material with chloroform using a Soxhlet apparatus. All extracts were rotary evaporated. In order to obtain minimum inhibitory concentration (MIC) values agar diffusion method was used and inhibition halo diameter was determined.

Results: MIC values have a significant difference ($P < 0.05$) between plant extracts. Epazote and papalo extracts inhibit *B. cereus* at 200 and 800 mg/l, respectively. *S. aureus* was inhibited by the three extracts at 200 mg/l; moreover, it was the more sensitive microorganism with inhibition halo diameter (11.6+0.23 cm) that represents a 44% of inhibition.

Significance: All plant extracts tested have significant antibacterial activity against *B. cereus* and *S. aureus*, the extent of their potential use as agrochemicals requires further examination.

P3-28 Extended-spectrum β-lactamase(s) Gene Transfer of *Klebsiella pneumoniae* in Raw Foods

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Introduction: Raw foods such as vegetables and unpasteurized milk may contain commensal bacteria harboring antibiotic resistance genes, such conditions increase the potential for antibiotic resistant gene transfer. *Klebsiella pneumoniae* is common commensal bacteria associated with raw agricultural products. Extended-spectrum β-lactamases (ESBLs) gene mainly are encoded on conjugative self-transferable plasmids, contributing to the rapid spread of ESBLs producing *K. pneumonia* into the community setting.

Purpose: The objective of this work was to investigate the potential spread of ESBL genes in raw foods.

Methods: *K. pneumoniae* (n = 8) were isolated from 17 different packages of retail seed sprouts. Four of the 8 isolates and KP342 (origin from maize) were used as recipients. ESBL-producing clinical (ATCC 700603) or bovine *K. pneumoniae* were used as donor isolates. Based on their profile of virulence genotype and antibiotic resistance phenotype, a donor and recipient were mated in TSB, unpasteurized milk (UPM), pasteurized milk (PM), and alfalfa sprouts (AS) at defined temperatures. Transconjugants were verified by amplification of isolate specific virulence genes and transferred ESBL genes by PCR.

Results: All isolates from seed sprouts were resistant to ampicillin. However, none of those isolates were ESBL positive. Gene transfer in TSB, UPM, and PM occurred at 24°C (room temperature) and 37°C, but not at 4°C. The bla_{SHV18} gene of ATCC 700603 was transferred at a frequency of 6.4E-07 to 4.0E-04 in those mating. The bla_{CTX-M} gene of bovine isolates was transferred to KP342 at a frequency of 6.4E-07 to 3.23E-05 depending on the medium. The transfer rate in alfalfa sprouts was significantly higher than in TSB, UPM, and RM at 24°C ($P < 0.05$). However, there was no significant difference between 24°C and 37°C.

Significance: This work shows evidence that raw foods which carry high numbers of commensal bacteria can serve as a matrix for ESBLs gene transfer from *K. pneumonia.*

P3-29 Synergistic Effects of Essential Oils and a Plant Extract against Multi-drug Resistant *Salmonella enterica* on Organic Leafy Greens

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Developing Scientist Competition

Introduction: The frequent outbreaks associated with leafy greens suggest the need for improved antimicrobial treatments. Plant essential oils are well known for their antimicrobial activity. However, one concern with regard to the use of these oils is that effective antimicrobial concentrations may adversely affect the sensory attributes of the treated product.

Purpose: The purpose of this study was to demonstrate the effectiveness of essential oils with other antimicrobials at lower concentrations in wash water against *Salmonella* on organic leafy greens.

Methods: Ten-gram samples of organic baby and mature spinaches and romaine and iceberg lettuces were inoculated with antibiotic-resistant *Salmonella* Newport and dip treated in (a) phosphate buffered saline; (b) 3% hydrogen peroxide; (c) 0.1% cinnamon oil and 3% olive extract combination; and (d) 0.1% oregano oil and 3% olive extract combination. Samples were collected on days 0, 1, and 3 for enumeration of survivors.

Results: From an initial value of 5.0 and 5.3 log CFU/g, baby spinach showed 3.5 and 4.0 log CFU/g reductions on day 3 for the cinnamon oil plus olive extract and oregano oil plus olive extract combinations, respectively. Both combinations induced a 3.0 log CFU/g reduction in mature spinach. For Romaine lettuce, there was 3.0 log CFU/g reduction for the cinnamon oil and olive extract combination, and a 4.0 log CFU/g reduction for the oregano oil and olive extract combination. For iceberg lettuce, the cinnamon oil and olive extract treatment yielded a 2.75 log CFU/g reduction from an initial value of 5.1 log CFU/g, while the oregano oil and olive extract treatment yielded a 3.5 log CFU/g reduction.

Significance: The combination treatments were more effective against *S.* Newport than individual antimicrobials and, may also help retain the organoleptic attributes of organic leafy greens. Combination antimicrobials have the potential to serve as alternative sanitizer options.

P3-30 Minimum Lethal Concentrations of Essential Oil Components against Lactic Acid Bacteria

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Developing Scientist Competition

Introduction: Essential oils are gaining popularity as substitutes for previously utilized synthetic antimicrobial agents in the food industry. Consumer demand for industry transparency regarding additives and origins of their food products is the driving force behind what are perceived as more natural additives.

Purpose: Better understanding of the antimicrobial efficiency in terms of minimum lethal concentrations was examined to determine the applicability of essential oils against common food spoilage microorganisms.

Methods: Three genera of lactic acid bacteria, including *Leuconostoc citrovorum*, *Lactobacillus buchneri*, and *Pediococcus acidilactici*, were evaluated for survival up to 72h in de Man, Rogosa, Sharpe broth containing one of four essential oil components: carvacrol, cinnamaldehyde, eugenol, or thymol.

Results: Thymol was bactericidal (> 4-log reduction) against *L. citrovorum* at 1000 ppm and resulted in a 2-log CFU/ml reduction of *P. acidilactici* and > 2-log reduction of *L. buchneri*. Cinnamaldehyde caused a > 2-log reduction against all three species after 48h at concentrations ranging from 2000 to 2500 ppm. A > 3-log reduction was demonstrated for all three species after exposure to 2000 ppm carvacrol for 6 h. Eugenol at 2000 and 2500 ppm was required for > 3-log CFU/ml reduction after 6 h. *P. acidilactici* and *L. buchneri* recovered to initial inoculum concentrations after exposure to

2000 ppm eugenol by 48-72h. Eugenol at 3000 ppm caused a > 3-log reduction of *P. acidilactici* for the entirety of the 72h study period, but *L. buchneri* recovered to initial populations at the same concentration.

Significance: Essential oils at 1000-3000 ppm can inactivate common spoilage lactic acid bacteria, but regrowth over time may be a concern and may require multiple antimicrobial hurdles to achieve a desired shelf life in foods.

P3-31 Synergism between Florfenicol and Thiamphenicol against *Escherichia coli* and *Staphylococcus aureus*

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Introduction: Florfenicol (FF) and thiamphenicol (TAP) are amphenicol antibiotics commonly used in veterinary and aquaculture practice with broad-spectrum antimicrobial activity. Our previous study found that heating of FF produced TAP and the antimicrobial activity sustained even when 90% of FF is converted to TAP. Therefore, a synergistic effect between FF and TAP was proposed.

Purpose: To investigate the hypothesis that synergistic antimicrobial effect between FF and TAP exists for certain bacteria species.

Methods: A total of 57 field isolates including *Escherichia coli, Riemerella anatipestifer, Salmonella enterica*, and *Staphylococcus aureus* from avian, pig, cattle, dog and cat origins were collected. In addition, two ATCC strains, *E. coli* (ATCC 25922) and *S. aureus* (ATCC29213) were also used. The checkerboard microdilution assay and agar dilution assay were employed to determine the minimal inhibitory concentration (MIC) and synergism while time-kill curves were also applied to further validate the synergistic effect on susceptible strains.

Results: The synergistic effect was observed on 36% of *E. coli* isolates (n = 22) and 62% of *S. aureus* isolates (n = 13). The MIC of FF could be reduced by 87.5% (1/8 MIC) when combined with 1/2 MIC of TAP to exhibit antimicrobial activity comparable to the respective drugs at its original strength (1×MIC). The fractional inhibitory concentration indexes were ≤ 0.5 in 3 *S. aureus* strains and the synergism was further demonstrated by the time-kill assay. Some of the FF resistant strains also showed susceptibility to the synergistic combination. In contrast, the synergism was not observed in the 18 *Salmonella* and 4 *R. anatipestifer* strains tested.

Significance: This antibiotics synergism may offer potential alternatives for effective therapy against infections of these two pathogens. Food safety could be improved by reduced drug use and associated residues/toxicity at lower cost.

P3-32 Antimicrobial Efficacy of a Commercial Citrus Flavonoid and Acid Blend against Foodborne Microorganisms

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Introduction: Microbial control strategies are needed in the food industry to prevent foodborne illnesses/outbreaks and prolong product shelf life.

Purpose: The objective of this research was to investigate the antimicrobial efficacy of a commercial naturally-derived citrus flavonoid and organic acid blend (CFA) against the foodborne pathogenic and spoilage microorganisms, *Escherichia coli*, *Salmonella enterica*, *Enterobacter* spp., *Bacillus* spp. and *Staphylococcus aureus*.

Methods: The antimicrobial was added to bacterial cultures in broth at 7.8-125 ppm, and growth was monitored for 48h at 22°C. Apple juice and milk were used as model food systems to observe the commercial antimicrobial efficacy against tested microorganisms.

Results: At 22°C, CFA inhibited the growth of all tested microorganisms. Greater than a 1-log reduction of *S. aureus* and *B. cereus* was observed within the first 4h exposure at 7.8 and 31.25-ppm CFA, respectively, and inactivation to undetectable levels (>4.5-log reduction) was achieved at 31.25 ppm for both bacteria by 48h. CFA at 62.5 ppm extended the lag-phase of *E. coli* for up to 24h. At 125 ppm, CFA caused continuous reduction of viable *S. enterica* over 24h. For *E. aerogenes*, 31.25-ppm CFA resulted in 1, 2.5 and 4-log reduction after 8, 24 and 48h exposure, respectively.

In apple juice, 120-ppm CFA reduced viable *E. coli* and *Enterobacter* spp. by at least 2 log CFU/ml, while *S. enterica* was reduced to undetectable levels (>4-log reduction) in the presence of 180-ppm CFA in 48h at 22°C. When CFA was applied to milk at 150-18,000 ppm, no inhibition was observed in any tested microorganisms.

Significance: These findings suggest that the commercial CFA is an effective antimicrobial against the foodborne bacteria tested and has potential to enhance food safety and extending product shelf life of high carbohydrate, low fat and protein foods, such as fruit-based products.

P3-33 Efficacy Studies of Bromine-based Biocides for the Control of Microorganisms on Beef

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Introduction: Beef offers a favorable environment for growth of spoilage organisms and pathogens, therefore antimicrobials or other adequate technologies need to be applied to beef to ensure meat safety. Although bromine-based antimicrobials have been widely used in recreational and industrial water treatment for many years, they have only recently been approved for use in some food safety applications. Some advantages of bromine-base biocides are: their effectiveness at a wider range of pH values than chlorine, low odor and minimal effect on organoleptic properties.

Purpose: To evaluate bromine-based biocides BoviBrom® (1,3-Dibromo-5,5-Dimethyl hydantoin) and CellVex™ (NaBr+NaOCl) in both lab and in commercial beef plants as means to control bacteria in the beef industry.

Methods: In a laboratory study, inoculated fresh beef cutaneous trunci were sprayed with BoviBrom®. In a second study in a commercial beef plant, beef carcasses were sprayed with CellVex™. In a third study, beef carcasses were sprayed with BoviBrom® in a commercial beef plant.

Carcasses were swabbed using USDA's guidelines. Swabs were placed in Butterfield's buffer and this analyzed for total aerobic bacteria (APC) and Enterobacteriaceae (EB) using 3M™ Petrifilm™ incubated at 37°C for 24 or 48 hours

Results: Lab spray treatments of BoviBrom showed bacterial reductions up to 2.5 log CFU/cm^2 for APC and 2.1 log CFU/cm^2 for EB. CellVex treatment on beef in a commercial plant showed reductions of 1.66 log CFU/cm^2 for APC and a 1.01 log reduction of EB. BoviBrom treatment in a commercial beef plant showed a 1.72 and 1.35 log CFU/cm^2 reduction of APC and EB, respectively.

Significance: Bromine-based antimicrobials are an effective intervention to reduce bacteria and pathogens in the beef industry.

P3-34 Effect of Plant Proteases on Infectivity of Tulane Virus

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Introduction: Plant proteases, papain and bromelain, can be extracted from pineapple and papaya and have application in the meat, brewing, and dairy industries. Protein digestion by these enzymes has also yielded bacteriostatic, antifungal, antihelminthic, and antitoxin effects. Bromelain also cleaves influenza viral surface antigens to aid in vaccine development.

Purpose: The objective of this study was to evaluate the effect of papain and bromelain on Tulane virus (TV) as evaluated by TV infectivity in cell culture.

Methods: TV was propagated in LLCMK2 cells in the absence of serum, filtered (0.2 um pore) from cell debris, and treated in duplicate trials (approximately 10^4 PFU/ml initial concentration) for two hours at 37°C with papain at 1,000 ppm or a combination of papain and bromelain at 500 ppm each. The effect of protease treatment on TV infectivity in LLCMK2 cells was evaluated by plaque assay in cell culture media M199 containing 10% fetal bovine serum to inhibit plant protease activity on host cells. Controls included untreated TV, TV treated at 37°C for 2h without papain or bromelain, uninoculated cell culture media, and uninoculated protease solutions held at 37°C for 2h.

Results: No reduction in TV infectivity was observed as a result of treatment by papain and bromelain at the conditions tested. TV was also stable to the mild heat treatment in the absence of plant proteases. Detection sensitivity was at least 10 PFU/ml.

Significance: Plant proteases, especially those with current applications in food processing, would make desirable antiviral agents for enhanced safety of raw produce and ready-to-eat foods. While the conditions tested did not reduce TV infectivity, additional treatment conditions to enhance enzymatic activity or in combination with other sublethal stresses are worthy of investigation.

P3-35 Antimicrobial Efficacy of Fulvic Acid Formulations against *Escherichia coli* O157:H7 on Bagged Organic Leafy Greens at Refrigeration Temperatures

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Introduction: *Escherichia coli* O157:H7 is a public health problem worldwide, with leafy greens as a major source of recent outbreaks. Standard organic-produce-industry washes have been ineffective in reducing this problem. Need for effective antimicrobials for organic produce is therefore clearly evident. Fulvic acid is an organic acid reported to have antibacterial and antifungal properties. Previous investigations have shown its effectiveness *in vitro* against a range of bacteria: *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis, Klebsiella pneumoniae,* and *Candida albicans*. To our knowledge, no studies have been conducted to determine its efficacy against *E. coli* O157:H7 in organic leafy greens.

Purpose: Determine the antimicrobial efficacy of fulvic acid against *E. coli* O157:H7 in organic leafy greens at refrigerated temperatures.

Methods: Organic baby and adult spinach, and romaine and iceberg lettuce were inoculated with a cocktail of three *E. coli* O157:H7 strains (10^6 CFU/ml). Fulvic acid I, II, III and IV at different concentrations (1, 2 and 3%) were prepared in sterile deionized water. The inoculated greens were washed in each treatment solution for 2 minutes, bagged and refrigerated at 4°C. Hydrogen peroxide and water were used as the controls. Surviving bacterial populations were enumerated on days 0, 1, and 3 of storage.

Results: Significant log reductions ($P < 0.01$) were observed in pathogen populations on leafy greens treated with all fulvic acid concentrations. Fulvic acid III was most effective, with a 3-log reduction for each leafy green by day 3. Fulvic acid II (3%) and III (1%) showed a 2.8 and 2.5-log CFU/g reduction, respectively, in romaine lettuce and 1.6 to 2.0-log reduction for the rest of the leafy greens. Fulvic acid I and IV resulted in 1.4 to 1.8-log CFU/g reductions, respectively.

Significance: Fulvic acid is an effective natural antimicrobial and can be used as a wash treatment for organic leafy greens stored at refrigerated temperatures.

P3-36 Inhibition of *Escherichia coli* O157:H7 and *Salmonella* Saintpaul Using Plant-derived Antimicrobial Essential Oils in Surfactant Micelles

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Developing Scientist Competition

Introduction: Plant essential oils (EO) possess antimicrobial activity against bacterial pathogens. Encapsulation of EO in surfactant micelles can provide enhanced inhibition of bacterial foodborne pathogens.

Purpose: The objective of this study was to identify the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different antimicrobial EO-containing micelles against foodborne pathogens.

Methods: Surfactants (Tween® 20, Surfynol® 485W, sodium dodecyl sulfate (SDS), and CytoGuard® LA20) were dispersed in sterile distilled water to different concentrations; eugenol and carvacrol (0.05-9.0% w/v) were added to surfactant solutions and stirred until completely emulsified. The micelle solutions were filter sterilized using a membrane filter (0.2 µm pore diameter). Antimicrobial activity of micelles was determined by addition of different concentrations of 2X micelle solutions to a 96-well plate. *Escherichia coli* O157:H7 and *Salmonella* Saintpaul in 2X tryptic soy broth (TSB; 5.0 log CFU/ml target inoculum) were then added to micelle-loaded wells. OD_{630} of test wells was measured before and after incubation at 35°C for 24 hr. After baseline adjustment, test wells showing <0.05 increase in OD_{630} were considered inhibitory; the lowest concentration of antimicrobial micelle producing pathogen inhibition was identified as the MIC for each EO/surfactant system. One hundred (100) µl from pathogen-inhibiting samples were spread on tryptic soy agar (TSA)-loaded Petri dishes and incubated for 24 hr at 35°C. The concentrations of micelle solution that produced ≥3.0 log CFU/ml reduction of the inoculated pathogen were classified as bactericidal; the lowest concentration of EO-micelle was deemed the MBC. All assays were performed in triplicate (n = 3).

Results: The lowest MIC of antimicrobial-bearing micelles against pathogens was observed in 0.5% SDS+ 0.1125% carvacrol while the lowest MBC was observed in 0.0625% CytoGuard LA20 + 0.0016% eugenol or carvacrol.

Significance: Antimicrobial micelle systems containing plant-derived EO may be useful delivery systems for the decontamination of fresh and/or fresh-cut produce.

P3-37 Effect of the Food Components Sodium Caseinate, Soybean Oil and Potato Starch on the Antimicrobial Efficacy of Trans-cinnamaldehyde and Eugenol against *Escherichia coli* BA-1882 and *Listeria monocytogenes* Scott A

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Introduction: Trans-cinnamaldehyde and eugenol are the main components of essential oils from cinnamon bark and cloves. Both have been shown to have antimicrobial activity against foodborne bacteria, however most studies are in microbiological media.

Purpose: The objective of this study was to quantitate the influence of a food protein, lipid and carbohydrate on the antimicrobial efficacy of cinnamaldehyde and eugenol. The model food components were sodium caseinate, soybean oil and potato starch.

Methods: Initially, the minimum inhibitory concentration (MIC) of cinnamaldehyde and eugenol against *Escherichia coli* (EC) BA-1882 and *Listeria monocytogenes* (LM) Scott A were determined at 37°C and 32°C, respectively, by the microbroth dilution method. Then, growth curves were performed at the MICs of the essential oils in tryptic soy broth (EC) or TSB plus yeast extract (LM). Food components were added at 0, 1% and 5% (w/v). Incubation was: EC: 37°C, 24h; LM: 32°C, 48h.

Results: The MICs for EC and LM, respectively, of cinnamaldehyde were 250 and 150 ppm and of eugenol 500 and 550 ppm. For EC, cinnamaldehyde antimicrobial activity was reduced significantly by 1% soybean oil and eliminated by 5% oil. In contrast, 1% caseinate and starch had little effect on activity. For eugenol, the negative effect was more pronounced with 1% of all significantly reducing activity. For LM, results were similar for soybean oil but caseinate and starch had much less of an effect on antimicrobial activity, even at 5%.

Significance: These results indicate that food components reduce, but do not always eliminate, antimicrobial activity. Food components had less of an effect on cinnamaldehyde than eugenol. Thus, cinnamaldehyde has potential for activity in foods with higher protein, higher starch or moderate to low lipid content. Eugenol has much less potential to be incorporated into foods without a significant loss of antimicrobial activity.

P3-38 Inhibition of *Listeria monocytogenes* with a Lactic Acid Bacteria Treatment for Fresh Strawberries

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Introduction: Approximately 600 outbreaks of illness associated with fresh produce have occurred since 1990. *Listeria monocytogenes* may contaminate fresh produce commodities such as strawberries so it is necessary to investigate interventions such as lactic acid bacteria (LAB) to inhibit this pathogen.

Purpose: The purpose of this study was to evaluate different combinations of LAB strains to inhibit *L. monocytogenes* on fresh strawberries.

Methods: Approximately three pounds of fresh strawberries were inoculated with a cocktail of *L. monocytogenes* strains (Scott A and Brie) at less than 4.00 log CFU/g. A portion of strawberries (25%) were reserved as an inoculated control (not treated) and the remaining strawberries were divided into the following LAB treatments, each applied as a dipping: 1) *Lactococcus lactis* FS56, 2) *Lactobacillus acidophilus* NP51 and *Lactobacillus plantarum* C28, and 3) *L. lactis* FS56, *L. acidophilus* NP51, and *L. plantarum* C28. After treatment, the strawberries were stored at 4°C with samples collected on days 0, 1, 3, and 7 to enumerate *L. monocytogenes* by plating on Modified Oxford agar. Microbial counts were log transformed. A repeated measures ANOVA was used to analyze the results.

Results: There was a significant treatment effect ($P = 0.005$) and the strawberries treated with 1) *L. lactis* FS56, 2) *L. acidophilus* NP51 and *L. plantarum* C28, and 3) *L. lactis* FS56, *L. acidophilus* NP51, and *L. plantarum* C28 all had a significant lower *L. monocytogenes* populations when compared to the control group.

Significance: These data suggest that the LAB treatments can serve as an effective intervention to reduce *L. monocytogenes* on fresh strawberries and will improve the safety of the product.

P3-39 Aqueous Methanol Extracts of Pomegranate Peel and Chinese Gallnut Inhibited Growth of *Vibrio parahaemolyticus* and *Listeria monocytogenes*

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Developing Scientist Competition

Introduction: Traditional Chinese medicines (TCM) have been widely studied as complementary and alternative medicines, and many of them have been verified to have antimicrobial properties. *Vibrio parahaemolyticus* and *Listeria monocytogenes* are important foodborne pathogens associated with Ready-to-Eat (RTE) seafood products.

Purpose: The purpose of this research was to study antimicrobial effects of plant extracts as potential preservatives in seafood products and to identify the primary antimicrobial compounds in plant extracts.

Methods: Four plants, Pomegranate peel ("PP", *Punica Granatum* L.), Chinese gallnut ("CG", *Galla chinensis*), forsythia ("FS", *Forsythia suspensa*) and Baikal skullcap ("BS", *Scutellaria baicalensis*) were ground and extracted with 70% methanol, respectively. Five concentrations of each extract (0.008, 0.04, 0.2, 1.0, and 5.0 mg/ml) were applied to *V. parahaemolyticus* and *L. monocytogenes* cultures which were then incubated at 37°C, and sampled after 8, 24, and 96 hours. Samples were spread plated, incubated and enumerated to determine the level of inhibition by plant extracts. Extracts from each plant were analyzed by HPLC, and then fractions were collected based on elution time and tested for their antimicrobial activity against *V. parahaemolyticus* and *L. monocytogenes* using agar diffusion methods.

Results: Both CG and PP extracts, with concentrations no lower than 1 mg/ml, significantly inhibited both pathogens by up to 4 logs. No inhibition was observed with FS and BS extracts, except for BS at 5 mg/ml against *V. parahaemolyticus*. Inhibitory activities of CG and PP extracts were distributed among all fractions, thus in each plant extract more than one compound contributed to inhibition, most of which were categorized as flavonoids, hydrolysable tannins and their pentosides or hexosides.

Significance: Traditional Chinese medicine plant-source antimicrobial compounds can inhibit foodborne pathogens including *V. parahaemolyticus* and *L. monocytogenes*, and therefore can be potential natural preservatives for RTE foods.

P3-40 Tryptophan Acts as an Incompatible Solute: Growth Inhibition of *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* O157:H7

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Introduction: Bacterial cells uptake compatible solutes, such as glycine betaine, under osmotic stress to maintain homeostasis. However, there might be incompatible solutes that are similar in structure to compatible solutes but have different physiological effects on bacterial growth. These incompatible solutes could be used in foods as alternative preservatives.

Purpose: The purpose of this study was to evaluate tryptophan as an antibacterial substance inhibiting the growth of pathogenic bacteria under ambient temperature.

Methods: *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 were evaluated. The bacterial growth was monitored at 25°C in peptone-yeast-glucose broth supplemented with 0 to 4% (w/v) salt and tryptophan (D/L/DL) in concentrations between 0 and 40 mM. After the broth was inoculated with pathogens, the optical density at 595 nm was continuously monitored every 10 min during the incubation period. Growth curves were generated, and time-to-detection and specific growth rates were calculated and compared between the tested conditions using the Tukey's multiple comparison test.

Results: D-tryptophan greatly inhibited all bacterial growth tested in this study. In particular, D-tryptophan completely inhibited the growth of *E. coli* O157:H7 and *Salmonella* in the presence of more than 3% salt over a 3-day incubation period. The salt concentration in the environment greatly influenced the antibacterial effect of D-tryptophan. In the culture with no added salt (0% NaCl), all pathogens exhibited significant growth inhibition with 40 mM D-tryptophan. However, 1-2% NaCl did not significantly affect bacterial growth, while more than 3% NaCl conditions caused a significant impact on the inhibition of the bacterial growth.

Significance: The antibacterial effect of D-tryptophan demonstrated in this study could be an alternative approach to controlling bacterial growth in foods.

P3-41 Cocoa on Growth of Major Enteric Bacterial Pathogens and Their Interaction with Intestinal Epithelial Cells

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Developing Scientist Competition

Introduction: *Salmonella enterica* serovar Typhimurium (ST), enterohemorrhagic *Escherichia coli* (EHEC), and *Listeria monocytogenes* (LM) are three of the major common foodborne bacterial pathogens in the US and they are responsible for most foodborne infections. Due to the rising of multi-drug resistant bacterial pathogens, finding of natural alternative and consumer friendly antimicrobials is essential. It has been suggested that cocoa might be one of them based on its rich sources of antioxidants (e.g., flavanols) and various other free radical scavenging molecules.

Purpose: The purpose of this study was to evaluate the antimicrobial property of cocoa against EHEC, ST and LM and its role in interactions of these foodborne bacterial pathogens with intestinal epithelial cells.

Methods: The growth conditions of *Salmonella* Typhimurium LT2 (ATCC19585), enterohemorrhagic *E. coli* EDL933 (ATCC700927), and *Listeria monocytogenes* LM2 (ATCC19115) were compared between in broth with and without 3% cocoa powder. The adhesion and invasion abilities of the bacterial strains into INT-407 cell with and without 3% cocoa powder were also examined. Comparisons between treatments and control were performed using ANOVA.

Results: 3% cocoa reduced the growth of EHEC, ST, and LM significantly ($P < 0.05$) with the maximum effects within first 3 hours of incubation. In addition, cocoa powder significantly ($P < 0.05$) inhibited adhesion to and invasion of INT-407 cells by these bacterial pathogens in a dose-dependent manner. For instance, in the presence of 3% cocoa powder, growth of EHEC, ST, and LM were reduced 1.58, 1.60, and 0.25 logs CFU/ml, respectively. Likewise for adhesion abilities (reduced by 73.0%, 76.5%, and 37.7%, respectively), and invasion abilities (reduced by 89.8%, 100%, and 100%, respectively).

Significance: The antimicrobial effects as well as alteration of host-pathogen interactions suggests that cocoa may aid in the prevention of foodborne illness caused by major foodborne enteric bacterial pathogens including EHEC, ST, and LM.

Poster

P3-42 Laboratory Examination of Lactic Acid and Peroxyacetic Acid as Antimicrobial Applications for Whole, Fresh Apples

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Introduction: Identifying effective antimicrobial interventions to reduce foodborne pathogen risk is important for the fresh apple packing industry

Purpose: Laboratory inoculation studies with pathogenic and generic *Escherichia coli* on whole, fresh Gala apples examined antimicrobial activity of lactic acid (LA) and peroxyacetic acid (PAA).

Methods: Cocktails of generic *Escherichia coli* (*E. coli*) (ATCC 25922, 11755, 23716) or *E. coli* O157:H7 (43890, 43895, SEA13B88) were surface inoculated by massaging apples for 10 minutes. For each replication (n = 3), 220 fresh gala apples (660 total) were used. Lactic acid (1% and 2%) and water treatments were examined at application times of 5, 15 and 30 seconds to mimic commercial spray bar applications. PAA (60 and 80 ppm) and water treatments were examined at application times of 2, 3.5 and 5 minutes to mimic commercial flume applications. Samples were enumerated on violet red bile agar for generic *E. coli* and Cefixime-Tellurite sorbitol-MacConkey agar for *E. coli* O157:H7.

Results: Bacterial levels on apples treated with water and LA (1% and 2%) were significantly ($P < 0.05$) lower than the inoculated control (1 log CFU/ml reduction. Application time and the concentration of LA did not influence microbial reductions, and levels of pathogenic and generic *E. coli* were similar between apples treated with water and LA. For 60 ppm PAA at 3.5 and 5 minutes and for all application times at 80 ppm significant bacterial reductions ($P < 0.05$) were achieved compared to the water treatments and the inoculated control treatment (average 1.5 log CFU/ml reduction).

Significance: PAA treatments of 80 ppm for 2-5 minutes and 60 ppm for 3.5-5 minutes reduced microbial levels sufficiently for consideration as a dump tank or flume application for fresh apple packing.

P3-43 Surfactant Enhanced Disinfection of the Human Norovirus Surrogate, Tulane Virus, with Organic Acids and Surfactant

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Introduction: Human infection with foodborne viruses can occur following consumption of contaminated food, person-to-person body contact, or release of aerosols. Combinatorial treatments of surfactants and organic acids may have synergistic or additive mechanisms to inactivate foodborne viruses and prevent outbreaks.

Purpose: The purpose of this experiment is to investigate the efficacy of treatments of lactic (LA) or acetic acid (AA) in combination with sodium dodecyl sulfate (SDS) against a human norovirus surrogate, Tulane virus (TV).

Methods: An aqueous 4% (v/v) stock solution of LA or AA with 5.6% (v/v) SDS was adjusted to pH 2, 4 or 7 with approximately 0.5 ml of 0.1M NaOH. The stock solution was diluted to 2x of its desired concentration with growth media (M119 + 10% FBS). The solution was inoculated with 6 log PFU/ml of TV with a treatment time of 5 minutes. Because of the capacity of the growth media, pH of the final solution was measured after the treatment time. The survival of TV was quantified using a plaque assay with LLC-MK2 cells.

Results: The minimum concentration of treatments that produced significant ($P < 0.05$) log reduction of TV was 0.5/0.7% (v/v) LA/ SDS at pH 3.5 (4.5 PFU/ml reduction), 1.0/1.4% (v/v) LA/SDS at pH 4.2 (2.2 log PFU/ml reduction), and 0.5/0.7% (v/v) AA/SDS at pH 4.0 (2.6 log PFU/ml reduction). The combinatorial treatment of AA or LA with SDS at pH > 5 did not produce significant log reduction. No log reduction was observed with treatments of either AA, LA, or SDS alone at pH 2.

Significance: This experiment demonstrates that surfactants like SDS aid in the organic acid toxicity against viruses. However, inactivation of TV by combinatorial treatments is contingent upon the sanitizing solution's pH being below the pKa of the organic acid being used. This information can be used to develop sanitizing washes to disinfect food contact surfaces.

P3-44 Efficacy of a Citric Acid-based Organic Sanitizer against *Salmonella enterica* and Background Microflora on Celery and Leeks

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Introduction: The increased demand for fresh produce necessitates a reduction in microbial load post-harvest. Treatment with sanitizers is an effective way to reduce the pathogenic and background microorganisms on fresh produce.

Purpose: The objective of this study was to investigate the efficacy of a citric acid based organic sanitizer (organic Chicowash™) against *Salmonella enter*ica serovar Newport and background microflora on celery and leeks.

Methods: Three different concentrations (1:10, 1:20 and 1:40) of Chicowash solutions were used. De-ionized water and 200 ppm chlorine were used as controls. For background microflora testing, 10 g fresh cut celery or leeks were weighed for each sample. For *Salmonella* testing, produce samples were washed, dip inoculated in 10^7 CFU/ml *Salmonella* Newport culture and allowed to dry for 30 min to facilitate bacterial attachment. Samples were immersed in the sanitizer solutions for 2 min. Samples were taken for enumeration of survivors after storage at 4°C for 0, 1, 3 and 6 days.

Results: At day 6, Chicowash reduced *Salmonella* and background microflora population by 0.7-1.5 and 1.2-2.6 logs CFU/g, respectively. All three concentrations showed 0.3-1.0 log additional reduction in *Salmonella* population on celery and leeks than 200 ppm chlorine. At day 1, Chicowash showed 0.8-1.3 logs reduction in *Salmonella* population. At day 3, the reductions in *Salmonella* population were 1.3-1.6 and 0.7-1.6 logs on celery and leeks, respectively. At day 0, after Chicowash 1:10 treatments, the background flora population on leeks were 1.8 logs lower than that of 200 ppm chlorine treated samples. At day 6, background microflora population on celery and leeks samples were 1-1.7 and 1.5-2.2 logs less, respectively, than that on chlorine treated samples. The antimicrobial activity of Chicowash was concentration and storage time dependent.

Significance: The results could provide the fresh-cut produce industry with additional options to select organic sanitizers for post-harvest treatments.

P3-45 A Commercial Antimicrobial Packaging System of Ground Beef Based on "Tsipouro" or Distillery Ethanol

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Developing Scientist Competition

Introduction: The high popularity and perishability of ground beef motivates the food industry to apply modern preservation methods like antimicrobial packaging.

Purpose: Evaluation of the antimicrobial effect of distillery ethanol and 'tsipouro' through diffusion and/or evaporation on ground beef stored in commercial packages.

Methods: Different concentrations of ethanol (38, 70, and 100% v/v) and 'tsipouro' (Greek alcoholic distillate) were applied as antimicrobials in packages of ground beef (500g). Antimicrobials were added in absorbent cloths (17x12mm; 17x24mm), which were placed underneath beef and soaked with 20, 30, 40, and 60 ml of antimicrobials. Moreover, cloths (7x10mm) supplemented with 10 ml of the respective antimicrobial were also mounted under the packaging film of each package. Packages were stored under 80%O_2:20%CO_2 at 7°C. Untreated samples served as controls. TVC, pseudomonads, *B. thermosphacta*, lactic acid bacteria, pH, color (*L*,a*,b*),* and odor were evaluated. The migrated ethanol to the ground beef was estimated by GC-FID. Measurements were performed on samples taken from the top and bottom of the package to investigate potential differences regarding the antimicrobial efficacy and migration of antimicrobials through evaporation and/or diffusion.

Results: *B. thermosphacta* dominated the microbial association of meat. Growth of all studied microorganisms was delayed by increasing the applied volume and concentration of ethanol/'tsipouro'. For instance, on day 3, when TVC on controls reached 7.5 log CFU/g, samples treated with 'tsipouro' followed the order: 6.0 log CFU/g (60 ml) > 6.4 log CFU/g (40 ml) > 6.7 log CFU/g (30 ml) > 7.0-7.3 log CFU/g (20 ml) resulting in 2-5 days shelf-life extension. Ethanol and 'tsipouro' also inhibited pseudomonads (by 1.0-2.0 log CFU/g) compared to controls. Gradual decrease of *L** and *a** was observed on controls, while samples treated with ethanol (especially 38%) and 'tsipouro' showed more stable, red color. With regards to GC, ethanol (component) was higher ($P \leq 0.05$) on the bottom (diffusion) of the ground beef compared to the top (evaporation).

Significance: Such preservation methods may raise new perspectives on mild antimicrobial packaging in order to extend shelf life of perishable food products.

P3-46 Survival and Heat Resistance of *Escherichia coli* O157:H7 in Ground Pork as Affected by Nisin, Lysozyme, Oregano Essential Oil and EDTA

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Developing Scientist Competition

Introduction: The combined use of essential oils with chelators may enhance the antimicrobial activity of bacteriocins or lysozyme against Gram-negative pathogens.

Poster

Purpose: To investigate the impact of nisin, lysozyme, oregano essential oil and EDTA, singly or in combinations, on (i) the survival of *Escherichia coli* O157:H7 during refrigerated storage of ground pork and (ii) the subsequent thermotolerance of the pathogen.

Methods: Ground pork samples were formulated with (A) no antimicrobials, (B) 0.1% or 0.2%v/w oregano essential oil (O.E.O.), (C) 50mM EDTA, (D) nisin (500 or 5000 IU/g) + 0.1% or 0.2%v/w O.E.O, (E) lysozyme (500 or 5000 IU/g) + 0.1% or 0.2%v/w O.E.O (F) nisin (5000 IU/g) + 0.2%v/w O.E.O. + 50mM EDTA, (G) lysozyme (5000 IU/g) + 0.2%v/w O.E.O. + 50mM EDTA, (H) nisin (5000 IU/g) + lysozyme (5000 IU/g) + 0.2%v/w O.E.O, and (I) nisin (5000 IU/g) + lysozyme (5000 IU/g) + 0.2%v/w O.E.O +50mM EDTA. All samples were inoculated (10^6 CFU/g) with a 3-strain composite of *Escherichia coli* O157:H7, vacuum packed and stored at 4°C for 12 days. On day 0, 6 and 12, samples (A), (H) and (I) were heat treated at 64°C in a water bath (n = 3 per sampling point), in order to calculate the D-values of the pathogen.

Results: All antimicrobial combinations (B to I) reduced ($P < 0.05$) the population of the pathogen by 1.1 to 2 log CFU/g during refrigerated storage for 6 days, while no further decrease was observed until day 12. The addition of O.E.O. or EDTA increased the antimicrobial effect of nisin or lysozyme during storage. Following heat treatment, the pathogen showed lower D-values (0.22-0.33 min) in samples with all antimicrobials (I) compared with (H) and control samples (0.47-0.54 min). Treatment (H) also showed shorter shoulder period (0 – 1 min) than control (1.25 – 1.5 min), suggesting that these treatments sensitized the pathogen during storage and reduced its subsequent thermotolerance.

Significance: Our findings may assist in determining the proper antimicrobial formulations in ground pork products in order to maximize their safety against *E. coli* O157:H7 in case of accidental undercooking.

P3-47 Prevention of Biofilm Formation on Stainless Steel by Nanoscale Plasma Coating

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Developing Scientist Competition

Introduction: Biofilm formation is a problem of great importance in the food industry because, once formed, the microbial cells are much harder to remove. Low temperature plasma coating technology is a novel and effective method to prevent biofilm formation.

Purpose: The aim of this study was to investigate the anti-biofilm efficacy of nanoscale plasma coating of trimethylsilane (TMS) to prevent biofilms of important foodborne pathogens from forming on stainless steel which is widely used in food processing.

Methods: Stainless steel wafers (1 cm × 1 cm) were coated with TMS plasma to an approximate thickness of 30 nm using direct current power supply, and *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* each was allowed to form biofilms on the wafers for 48h. The biofilms were removed by rinsing and ultrasonicating each wafer four times for 30s each time and the pour-plate method was conducted to determine bacterial counts. In addition, the efficacy of TMS-coated wafers in combination with a sanitizer was also tested by dipping the wafers in a Quat solution for 15s and rinsing before sonicating and plating.

Results: The number of *L. monocytogenes* dropped from 10^7-10^8 to 10^4-10^5 CFU/wafer, on average. *E. coli* O157:H7 and *S. aureus* showed a 1-log reduction (10^8 to 10^7 CFU/wafer) in numbers. With the combined use of plasma coating and sanitizer, a 100% kill of *L. monocytogenes* was achieved, while for the sanitizer-only group, as many as 10^5 CFU/wafer remained.

Significance: Results of this study show that TMS-coating deposited on stainless steel surfaces could significantly reduce biofilm formation. Moreover, when combined with sanitizers, TMS coating shows greater promise to remove and prevent biofilm from forming on food contact surfaces.

P3-48 Purification of Microcin N – A Bacteriocin Effective against *Salmonella enterica* and *Escherichia coli*

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Developing Scientist Competition

Introduction: *Salmonella enterica* and *Escherichia coli* O157:H7 are foodborne pathogens commonly associated with disease outbreaks from the consumption of meat. Microcin N (McnN), a bacteriocin produced by a non-pathogenic strain of *E. coli* MC4100 pGOB18, has potential to control the growth and survival of these enteric pathogens. Purification of MccN for use in food systems has not been previously done.

Purpose: The objective of this study was to purify stable peptides for use as an antimicrobial in meat.

Methods: *E. coli* MC4100 pGOB18 was grown in minimal media and the production of McnN was confirmed with a deferred inhibition assay using *S. enterica* and *E. coli* as the indicator organisms. The supernatant of an overnight culture was passed through an Amberlite XAD16N column and the peptide eluted with 80% isopropanol containing 0.1% trifluoroacetic acid. Antimicrobial activity was confirmed by spot-on-lawn assay using *S. enterica* as the indicator organism and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to determine the presence of McnN. High performance liquid chromatography with a C18 column was used to further purify the active compounds. Fractions were concentrated and tested for antimicrobial activity using a spot-on-lawn assay. Active fractions were analyzed using MALDI-TOF MS to confirm the presence of peptides.

Results: After column purification, antimicrobial activity against *S. enterica* and *E. coli* was detected. MALDI-TOF MS revealed the presence of a peptide with a mass similar to that of McnN (7.3 kDa). Further purification with HPLC resulted in activity in spot-on-lawn assays but MALDI-TOF MS did not reveal the presence of a peptide with a mass consistent with McnN. However, smaller peptides were present.

Significance: Partial purification of McnN with column chromatography may provide a crude extract that could be used to control target pathogens in meat. Further research is required to obtain pure McnN.

P3-49 In-plant Validation and Microbial Performance of Peroxyacetic Acid as an Effective On-line and Off-line Reprocessing Treatment

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Introduction: There is increasing market demand for poultry products abroad as well as growing domestic demand for organic products. Processors have few processing aids acceptable for use on product processed for both export and organic markets.

Purpose: The purpose of this study was to validate the effectiveness of peroxyacetic acid (PAA) for use in on-line (OLR) and off-line (OFLR) reprocessing to reduce microbial populations on contaminated poultry carcasses.

Methods: In-plant trials were conducted on eighty carcasses identified by USDA FSIS as visually contaminated (VCO) and visually non-contaminated (VCL). Carcasses were processed through the plant's normal OLR or OFLR processing system containing PAA (25-100 ppm). Carcass samples were rinsed (1 min, 400 ml buffered peptone water); rinsates pH neutralized with sodium thiosulfate, and analyzed for generic *E. coli,* aerobic bacteria (APC),

Salmonella, and *Campylobacter*. Microbiological data were analyzed using Minitab Two-Sample *t*-Test and means separated using Tukey's method (α = 0.05).

Results: Findings demonstrate that PAA is an effective OLR and OFLR antimicrobial treatment to reduce APC, *E. coli*, *Salmonella* spp., and *Campylobacter* mean populations on poultry. APC and *E. coli* counts were significantly reduced ($P < 0.05$) from 4.5 ± 0.3 to 4.0 ± 0.4 and 3.1 ± 0.6 to 2.4 ± 0.8 log CFU/ml, respectively, on VCL carcasses and from 4.4 ± 0.5 to 3.3 ± 0.7 and 3.1 ± 0.8 to 2.4 ± 0.8 log CFU/ml on VCO carcasses through OLR treatment. *Salmonella* and *Campylobacter* were also reduced on carcasses treated on-line and off-line with PAA. Interestingly, *E. coli* and APC mean counts (VCO) measured at OFLR were surprisingly similar to those for OLR considering trials were conducted several months apart. This suggests that OLR systems treated with PAA could be suitable for reprocessing heavily contaminated carcasses eliminating required OFLR.

Significance: These data and application parameters can be used to support and validate PAA as an OLR and OFLR antimicrobial permitted for organic and export poultry products.

P3-50 Use of a Low pH Acid Solution for the Reduction of Bacteria on Baby Carrots

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Introduction: Food safety and reduction of bacteria on food products to increase shelf life is an important aspect to the food industry. Low pH acid solutions have been used in the food market with varying success.

Purpose: This study was designed to look at the application of a low acid pH solution (sold under the trade name Citrilow™) to baby carrots stored refrigerated and then tested on three different days.

Methods: Baby carrots were treated for 30 seconds by dipping the product into an acid solution of pH 1.5. The carrots were rinsed, allowed to drain, individually bagged and held refrigerated (untreated controls that were water rinsed were packaged and stored the same way). The baby carrots (control and treated) were tested on day 0, day 3, and day 5 for aerobic plate count (APC) bacteria.

Results: On day 0, the control product had 6.21 logs of bacteria and the treated product had 4.53 logs of bacteria for an initial reduction of 1.95 logs of bacteria. Day 3 showed the control had 6.24 logs of bacteria and the treated had 4.49 logs of bacteria, for a reduction of 1.80 logs of bacteria. Day 5 showed the control had 6.92 logs of bacteria and the treated had 5.21 logs of bacteria for a reduction of 1.42 logs of bacteria.

Significance: The treated samples showed a reduction of APC counts on day zero which resulted in lower APC counts on subsequent testing days. This reduction of bacteria produces two beneficial results; one it can reduce pathogenic bacteria as well as APC bacteria, thus producing a safer product for the consumer. And two, the reduction of APC bacteria can increase the shelf life of the product, providing the customer with a safer product that can be stored longer and provide cost savings as well.

P3-51 Antimicrobial Activity and Physical Properties of Biopolymer Films Containing Supernatants of *Lactobacillus sakei* Growth

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Introduction: Consumers' demand for a reduction in food additives, mainly chemical preservatives, has promoted the search for several alternatives. Free cells' supernatants of *Lactobacillus* growth can be incorporated as natural antimicrobials in biopolymer films, mainly to control pathogen growth in foods.

Purpose: The purpose of this study was to evaluate the antimicrobial activity and physical properties of biopolymer films containing free cells' supernatants of *Lactobacillus sakei* growth.

Methods: Calcium caseinate CAS (3% p/v) was mixed with glycerol (2%) and free-cells' supernatants were produced by the growth of *L. sakei* NRRL B-1917 in MRS broth for 18h at 37°C. Films were obtained by casting. To determine films' antimicrobial activity, circular portions of the films (0.6 mm diameter) were placed on trypticase soy agar inoculated with 10^7 CFU/ml of *S.* Typhimurium, *L. monocytogenes, E. coli*, or *S. aureus*; plates were incubated (37°C) for 24h and then the inhibition halos were determined. Film thickness was measured with a micrometer; moisture was determined by weight loss, and water vapor permeability of films was measured following ASTM methodologies.

Results: The use of free cells' supernatants of *L. sakei* in biopolymer films displayed inhibition halos against *E. coli* (4.83 ± 0.01 mm), *L. monocytogenes* (4.61 ± 0.02 mm), *S.* Typhimurium (4.21 ± 0.02 mm), or *S. aureus* (4.84 ± 0.02 mm). Supernatant incorporation altered films' physical properties, increasing their thickness (0.255 ± 0.001 mm), decreasing moisture (14.27 ± 0.21%) and increasing water vapor permeability (2.01 ± 0.11 g mm/m^{2}d kPa) in comparison to control films.

Significance: Results suggest that use of free cells' supernatants of *L. sakei* growth, as natural antimicrobials could be an alternative to replace synthetic antimicrobials, due to observed antimicrobial activity against studied food pathogens.

P3-52 Zinc Oxide and Silver Nanoparticle Effects on Intestinal Bacteria

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Introduction: The application of engineered nanoparticles (NPs) for food safety is increasingly being explored. Zinc oxide (ZnO) and silver (Ag) NPs have significant potential for a wide range of applications, including being incorporated into food packaging to improve food safety. However, NPs may also pose potential risks to consumers if they migrate from food packaging to food matrices. In our previous studies, the toxicity of ZnO and Ag NPs on the intestinal bacteria, *Escherichia coli*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* was investigated. Numbers of all three treated cells were within 1 log CFU/ml less than that of the control. However, not much is known about the mode of action of ZnO and Ag NPs on bacterial cells.

Purpose: The objective of this study was to investigate the mode of action of ZnO and Ag NPs on *E. coli*, *L. acidophilus* and *B. animalis* using multiple analytical tools.

Methods: Multiple analytical tools included scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), UV spectroscopy and confocal fluorescence microscopy.

Results: SEM and TEM images and EDS demonstrated adherence of NPs to the bacterial cells. Morphological changes of some cells were observed, but many remained in normal shapes. UV absorbance results indicated no significant leakage of internal cellular contents due to NPs. Viability of bacterial cells, as assessed by the Live/Dead Bacterial Viability assay, showed that more viable than dead cells was present after treatment with NPs.

Significance: This study revealed that ZnO and Ag NPs have milder inhibitory effects on intestinal bacteria as compared to those on pathogenic microorganisms that have been previously reported. Further research is required to investigate the exact mechanisms of how the NPs affect bacterial cells.

P3-53 Method for Inhibiting Yeast Activity

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Introduction: Yeasts represent most common spoilage problem in low pH food matrices like beverages, sauces, dressings and some diary and ready-to-eat products. *Candida* (e.g., *C. albicans*), *Saccharomyces* (e.g., *S. cerevisiae*) and *Zygosaccharomyces* (e.g., *Z. bailii*) are representative spoilage species for this segment. Currently, the preservation system of benzoate and sorbate is the most common used. Due to concerns like decarboxylation of benzoate to benzene and their chemical image, the food industry is looking for alternative natural preservatives. This study shows two examples of alternatives for the current preservation system. Both are based on a tea extract combined with another antimicrobial component. Both combinations showed unsuspected good antimicrobial properties.

Purpose: The objective is to evaluate the antimicrobial efficacy of tea extract in combination with a fatty acid derivative (LAE) and a fermentation-based product (polylysine).

Methods: Model low pH broth was prepared using water, sucrose, malt extract and peptone and an acidifier. *Candida albicans*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cells spores were independently inoculated to have ca. 2-3 log CFU/ml counts in the model system with pH of 3.5. Treatments with ECGC from 0 - 1%, polylysine LAE 0 - 0.007 % and LAE 0 - 0.004 % in 10 equal concentration steps were prepared. The inoculated samples were stored at 25 °C for 72 hours, respectively, for optical density measurement. All the treatments were done multiple samples (n = 5). The yeast growth was measured using OD measurement at 595 nm.

Results: The *C. albicans, S. cerevisiae and Z. bailli* were inhibited by 9000, 5000 and 2000ppm of sole tea extract, respectively. *Only S. cerevisiae* was inhibited by the other components tested in the mentioned concentration ranges. However, when tea extract was combined with polylysine or LAE an unsuspected enhanced efficacy was observed. All organisms were inhibited when treated with a combination of 700ppm tea extract and 5ppm polylysine or 20ppm LAE ($P > 0.05$).

Significance: This research shows an unsuspected increase of antimicrobial efficacy of a natural antimicrobial in combination with a food grade fermentation based product or a food grade fatty acid derivative. This research provides food industry with a potential antimicrobial to replace current chemical preservatives.

P3-54 Antimicrobial Activity of Binary and Ternary Mixtures Using Vanillin, Citral and Potassium Sorbate in Laboratory Media and Fruit Purées

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Introduction: The increasing demand for reduced-additive food has encouraged the search for alternative antimicrobial agents or combinations to be used by food industry

Purpose: The purpose of this study was to evaluate susceptibilities of *Escherichia coli* ATCC 35218, *Salmonella* Enteritidis MA44*, Saccharomyces cerevisiae* KE 162 and *Zygosaccharomyces bailii* NRRL7256 to mixtures of potassium sorbate (KS), vanillin (V) and citral (C) in laboratory media and agarized mango and melon purées (pH= 4.5, 13.0°Brix).

Methods: Antimicrobial mixtures were evaluated in laboratory media using the Berembaum experimental design. For each microorganism, fourteen systems were prepared using fractions of individual minimal inhibitory concentrations (MIC) previously determined (MIC_{KS}:150-5000 ppm; MIC_V:1000–3500 ppm; MIC_C:375–750 ppm). Systems were poured into 150 mm Petri dishes, swabbed with the inoculum, incubated up to 10 days at 30/37°C and daily examined for growth. Fractional inhibitory concentration (FIC) indexes and isobolograms were obtained. Several of these mixtures were validated in agarized fruit purees. Additionally, for some V/C combinations, KS inhibitory concentration was determined (0 ppm-512 ppm) by the spiral gradient endpoint (SGE) method.

Results: In laboratory media, inhibitory antimicrobial combinations were all additive (FIC index = 1), excepting some ternary mixtures that included fractions of antimicrobials between 1/12 MIC and 1/3 MIC which were synergistic (FIC index < 0.6) for the yeasts. For all microorganisms, all inhibitory Berembaum mixtures were confirmed in both agarized fruit purées.

When the SGE method was used (0 ppm < KS < 512 ppm), several inhibitory ternary mixtures were found (V < 1150 ppm; C < 250 ppm). The lowest values of KS concentration, for a given V/C combination, corresponded to bacteria in melon purée. *Z bailii* was not inhibited at any condition.

Significance: Some synergistic antimicrobial combinations could be useful in reducing the amounts to inhibit growth and diminishing consumer concerns about the use of preservatives.

P3-55 Incorporation of Nisin in Cellulose Casing of Bologna Sausage to Control Spoilage Microorganisms

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Introduction: Bacteriocins have emerged in the food industry as an alternative for food preservation. The incorporation of nisin in sausage casing could reduce the interaction of the bacteriocin with ingredients of meat products and control the growth of spoilage microorganisms

Purpose: The aim of this work was to evaluate the antimicrobial effect of nisin incorporated in cellulose casing on the population of lactic acid bacteria and total aerobic count in bologna sausage storage in vacuum packaging at 25°C.

Methods: Bologna sausages were produced in a pilot plant. The mixture of ingredients was stuffed into pretreated cellulose casings and the products were heat processed. After cooling the sausages were vacuum packaged and stored at 25.0°C. Cellulose casings were treated with nisin (1.0 $g \cdot l^{-1}$ Nisaplin®), at 40°C for 40 minutes, prepared in the following solutions: 1) potassium phosphate buffer (pH 6.0); 2) 0.1 % phosphoric acid (pH 2.0). As control, cellulose casings were also treated in the same solutions but without nisin. The population of lactic acid bacteria (LAB) and total aerobic count in bologna sausages were determinate during storage. The experiments were repeated three times and results analyzed using ANOVA.

Results: The treatment of cellulose casing with nisin solution (pH 6.0) reduced significantly ($P < 0.05$) the population of LAB and total aerobic count in bologna sausage by 1.7 log and 1.9 log at day 30 of storage and by 3.5 log and 3.7 log at the 50th day, respectively. However no inhibitory effect ($P > 0.05$) was observed when cellulose casings were treated with nisin solution in phosphoric acid (pH 2.0).

Significance: The results demonstrate that nisin (1.0 $g \cdot l^{-1}$) in potassium phosphate buffer solution (pH 6.0) can be incorporated in cellulose casing to reduce total aerobic count and lactic acid bacteria in vacuum packaged bologna sausage.

P3-56 Differences in Enterohemorrhagic *Escherichia coli* and *Salmonella* Prevalence in Raw Ground Beef and Trim

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Introduction: Beef producers routinely screen for the presence of enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella* in their products. While EHEC is found mainly on the surface of trim, *Salmonella* could be sequestered in the lymph nodes. Therefore, we might expect to find differences in the prevalence of these pathogens in different classes of beef products.

Purpose: To determine if there is a significant difference in prevalence over multiple years between EHEC and *Salmonella* in ground beef and trim.

Methods: From two processing plants that continuously tested trim and raw ground beef during calendar years 2010-2012, 336,771 trim samples (375g, 60 surface pieces) and 75,249 ground beef samples (375g) were enriched for a minimum of 9h and then screened by a multiplex PCR targeting the *stx* and *eae* genes of *E. coli* and two *Salmonella*-specific genes. Samples which were eae^+ and stx^+ (stx_1^+ and/or stx_2^+) were considered to be EHEC screen-positive. Samples sal_1^+ and/or sal_2^+ were considered to be *Salmonella* screen-positive.

Results: Over the three-year period, EHEC screen-positive sample prevalence was 8.15% and 11.38% in ground beef and trim, respectively, while the *Salmonella* screen-positive sample prevalence was 27.06% and 9.95% in ground beef and trim, respectively. All results are significantly different ($P \leq 0.05$).

Significance: The differences in prevalence of EHEC and *Salmonella* in ground beef and trim are consistent with the observation that the former is found primarily on the surface of beef trim (because hide is the primary source) while the latter is found in lymph nodes which are dispersed throughout ground beef as well as on the hides.

P3-57 Development and Application of the Rapidchek CONFIRM Immunomagnetic Separation (IMS) Kit for the Analysis of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) in Raw Beef

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Introduction: Worldwide, *E. coli* O157:H7 is the most common Shiga toxin-producing *E. coli* (STEC) associated with human illness. However, non-O157 STEC are becoming increasingly important and recently, the USDA FSIS has begun regulating serogroups O26, O45, O103, O111, O121, and O145 in raw beef as adulterants. Current methods for non-O157 STEC detection in raw beef are complex and ultimately require bacterial isolation and colony confirmation. In order to augment this, we have developed and applied immunomagnetic separation (IMS) reagents for the isolation and confirmation of non-O157 STECs from raw beef.

Purpose: The purpose of this study was to develop and apply new IMS reagents for the detection of non-O157 STEC in raw beef.

Methods: Affinity-purified polyclonal antibodies were developed against *E. coli* serogroups O26, O45, O103, O111, O121, and O145 and incorporated into IMS reagents. The IMS reagents were coupled to the USDA-FSIS modified TSB enrichment procedure for non-O157 STECs (MLG 5B.04) and this was followed by plating to both selective and non-selective agars and colony serotyping. This approach was evaluated for target non-O157 STEC recoveries in both mixed culture, where non-target STEC (O157) was present in 1 log excess of the target STEC (3 log), and coupled to non-selective agar plating, as well as in spiked raw beef (3 CFU target STEC/65 g) followed by enrichment and selective agar plating.

Results: In mixed culture experiments, before IMS, 9.4% of 360 colonies were identified as the respective non-O157 STEC whereas after IMS, 99.2% of 840 colonies were identified as the respective non-O157 STEC. In raw beef enrichments, before IMS, 54.9% out of 360 colonies were identified as the respective non-O157 STEC whereas after IMS, 99.2% out of 420 colonies were identified as the respective non-O157 STEC.

Significance: Application of the RapidChek CONFIRM IMS to the analysis of non-O157 STECs in raw beef products should streamline both food process and regulatory monitoring.

P3-58 In-plant Validation of Lactic Acid Spray as an Antimicrobial Treatment for Carcasses, Subprimals, and Beef Trim

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Introduction: Lactic acid spray is frequently applied to beef carcasses and subsequent cuts as an antimicrobial intervention to reduce *E. coli* O157:H7, *Salmonella*, and other pathogenic microorganisms that may be present.

Purpose: This in-plant validation study was conducted to determine the antimicrobial efficacy of lactic acid (2% to 5%) on indicator populations on cold beef carcasses, beef subprimals, and beef trim.

Methods: For each portion of the study (carcasses, subprimals and trim), 15 swab samples were collected before treatment and 15 samples were collected after treatment 3 times per day (beginning, middle and end of shift) A total of 180 samples for each product type were collected over two days. The swabs were then enumerated using petrifilms to determine aerobic plate counts (APC), coliform and generic *E. coli* (EC) populations per swab area. Reductions for each population were determined by comparing before and after treatment samples with an α-value of 0.1.

Results: Aerobic plate counts were significantly ($P < 0.0001$) reduced for those carcasses treated with lactic acid. Additionally, for subprimals, the intervention significantly reduced APC ($P = 0.046$) and coliforms ($P = 0.0197$) in the treated surfaces. When individual subprimal components were compared, APC populations for clods and top butts were significantly lower after lactic acid treatment. Coliform counts were also significantly reduced for clods, top butts and knuckles. No significant ($P > 0.1$) were noted for beef trim.

Significance: These data indicate that a lactic acid spray of 2% to 5% is effective in reducing indicator organisms commonly used by the industry to assess process control, thereby improving the safety of the product. This antimicrobial intervention can be applied to multiple product points within the beef processing facility.

P3-59 Biological Control of *Escherichia coli* O157:H7 Using Bacteriophage in Beef and Pork

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Developing Scientist Competition

Introduction: *Escherichia coli* O157:H7 is the most common foodborne bacteria in beef and pork. Application of bacteriophages as an emerging technology is getting attention to control foodborne pathogens in food safety worldwide.

Poster

Purpose: The purpose of this study is to examine the inhibition of *E. coli* O157:H7 in beef and pork using bacteriophage obtained from various sources.

Methods: Fourteen bacteriophages against *E. coli* O157:H7 were obtained from the bacteriophage bank in Korea. From the screening of lytic activity of bacteriophages, BEPCO 19 strain was selected. The meat samples with 2cm x 2cm were spiked on the surface of the each sample with *E. coli* O157:H7 10^5 CFU/sample. BEPCO 19 bacteriophage was treated on the surface with 1,000, 10,000, and 100,000 multiplicity of infection (MOI). All samples were stored at 4°C for 1, 2, 4, 8, 12, 24, 28, 72, 120, 168 hours. The populations of *E. coli* O157:H7 were measured on SMAC.

Results: The treatment of bacteriophage significantly reduced *E. coli* O157:H7 with MOI dependent manner. In beef, 1,000 and 10,000 MOI showed the 0.72 log and 2.35 log reduction until 48h and gradually increased. *E. coli* O157:H7 was not cultured in beef treated with 100,000 MOI after 8h. Although 1,000 MOI reduced 0.97 log reduction in pork until 12h, *E. coli* O157:H7 increased after 12h. Interestingly, *E. coli* O157:H7 was not cultured in pork treated with 10,000 and 100,000 MOI after 72h and 4h, respectively.

Significance: The treatment of bacteriophage effectively reduced *E. coli* O157:H7 in pork and beef. Control of *E. coli* O157:H7 by bacteriophage may be varied by the type of meat.

P3-60 Internalization and Post-cooking Survival of Non-O157 and O157:H7 Shiga Toxin-producing *Escherichia coli* in Blade Tenderized Beef Steaks

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Introduction: Blade tenderization is commonly used to increase palatability in beef products. However, the risk of pathogen internalization and post-cooking survival – particularly among serogroups of Shiga toxin-producing *Escherichia coli* (STEC) – in blade-tenderized beef is a concern for beef processors.

Purpose: This study investigated the effects of biochemical meat properties on the internalization and post-cooking survival of individual non-O157 and O157:H7 STEC serogroups in blade tenderized beef steaks.

Methods: Beef carcasses were selected to represent four USDA Quality Grade (QG; Choice or Select) × pH categories (dark cutter: 6.06 or non-dark cutter: 5.29 combinations. Strip loin subprimals from each carcass were divided into sections for inoculation (10^6 log CFU/cm^2) with one of seven individual STEC serogroups (O157:H7, O26, O45, O103, O111, O121, and O145). Subprimals were stored for 14 d prior to blade tenderization, steak portioning, and cooking to various endpoint temperatures (50, 60, 71, and 85°C). Pathogen presence was evaluated prior to and after storage and cooking.

Results: Neither carcass QG nor pH category influenced subprimal STEC attachment; however, STEC were greater ($P < 0.05$) on high pH steak surfaces. Greater than 3.5 log CFU/g of STEC were present in raw internal steak samples. Serogroups O121 and O45 were not found in cooked samples from any endpoint temperature. However, O26, O103, O111, O145, and O157:H7 STEC were confirmed in the internal cores of steaks cooked to 50°C and O26 STEC was confirmed in a cooked sample cooked to an endpoint temperature of 71°C. None of the seven STEC serogroups was confirmed in samples cooked to 85°C.

Significance: Blade tenderization promoted the internalization of STEC serogroups in non-intact beef products. Furthermore, the serogroups exhibited varied susceptibilities to commonly utilized endpoint cooking temperatures.

Poster

P3-61 A Comparison between Bovine Lymph Node Associated Isolates and Non-bovine Lymph Node Associated Isolates: An Intracellular Growth and Survival Assay within Bovine Macrophage Cells

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Developing Scientist Competition

Introduction: The incidence of human salmonellosis has remained unchanged when compared to 1996-1998 baseline data. While abattoirs implemented multiple hurdle interventions that have successfully reduced *Salmonella* contamination on beef carcass surfaces, *Salmonella* harbored in peripheral lymph nodes (PLNs) are not affected by these treatments and may re-introduce contamination during grinding.

Purpose: The purpose of this study was to determine the uptake, survival and persistence of *Salmonella* strains from human clinical cases and bovine PLNs in an immortal bovine macrophage cell line, a cell type found in PLNs.

Methods: Intracellular growth and survival of 32 *Salmonella* isolates, including isolates commonly or sporadically isolated from bovine PLNs and strains responsible for the majority of human disease, were probed in the BOMAC immortalized bovine macrophage cell line. Briefly, each strain was inoculated into duplicate confluent monolayers of BOMAC cells at a multiplicity of infection of 1,000:1. BOMAC monolayers were treated with gentamicin, washed and harvested at 0, 5, 9, and 12h post-inoculation. Resultant intracellular bacterial populations were enumerated by spread-plating on non-selective media. All strains were assayed in at least three biologically independent experiments and data were analyzed in mixed model.

Results: Statistical analysis revealed that source (human or bovine PLN) and serotype were significantly different ($P < 0.0001$), while time (0 to 12h) was marginally different ($P = 0.104$). Interestingly, human clinical isolates were taken up at a higher level and showed increased intracellular growth in BOMAC cells as compared to bovine PLN isolates. At 12h post-inoculation the mean intracellular population of isolates from human clinical cases was 6.09 log CFU/ml, while the mean bovine PLN population was 5.21 log CFU/ml.

Significance: These data show that *Salmonella enterica* serotypes isolated from bovine PLNs do not grow and survive inside of bovine macrophages as well as the common human serotypes do. Lower numbers may allow the bacteria to persist inside of the lymph node by not eliciting a large immune response.

P3-62 Hurdle Processing of Slight Acid Electrolyzed Water with Fumaric Acid for Bacterial Inactivation and Shelf-life Extension of Beef Meat from Slaughterhouse

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Developing Scientist Competition

Introduction: The consumption of foods contaminated with pathogenic microorganisms represents a wide economic and public health impact worldwide. Meat is a rich nutrient matrix that provides a suitable environment for proliferation of spoilage microorganisms and foodborne pathogens.

The consumption of contaminated meat represents a wide economic and public impact worldwide. Reduction of microbiological contamination levels in meat products could reduce the burden of foodborne disease and decrease economic losses and public health impact.

Purpose: The aim of this study was to investigate immediate and storage the hurdle processing effect of SAEW and FA on the inactivation of selected bacteria. In particular, quality decay of the investigated product was assessed by monitoring the following microbiological and sensorial quality of beef from slaughterhouse.

Methods: Inoculated meats were dipped for 1, 3, and 5 min immersed at 25, 40, and 60°C to evaluate the effect of temperature. The following dipping solutions were used either alone or in combination. Treated meat samples were treated were air-packaged using stomacher bag and storage at 4 or 10ºC. During storage, sampling was carried out at 2-day intervals for microbiological changes and sensory changes. A weighted sensory quality was calculated using sensory index equation. Data were subjected to analysis of variance ($P < 0.05$).

Results: Exposure time and temperature showed significant difference in sanitization potency against tested bacteria when compared to TW. Hurdle processing at 40°C exhibited a reduction of more 1.10 log units higher than the sum of the reductions obtained with the single treatments. And, except TAC, hurdle processing at 40°C showed a longer microbiological shelf extension regarding to those controls, since microbial load about 10^6CFU/g were achieved at 8 and 10 days for 4 and 10°C, respectively.

Significance: The combination of SAEW and FA could ensure the microbiological safety and quality of beef meat by efficacy contamination reduction and shelf life extension.

P3-63 Thermal Inactivation of Shiga Toxin-producing *Escherichia coli* within Cubed Beef Steaks Following Cooking on an Electric Skillet

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Introduction: Cubed steak is an eye round steak that has been mechanically tenderized to make it more desirable for the consumer. Since it is a non-intact product, it should be cooked like ground beef due to possible bacterial translocation into the deeper tissues of the meat as a result of the tenderization process. However, no studies have addressed the viability of Shiga toxin-producing *Escherichia coli* (STEC) in non-intact beef processed via mechanical cubing.

Purpose: Quantify thermal inactivation of STEC within knitted/cubed steak following cooking on an electric skillet.

Methods: For each of three trials, commercially-processed eye round steaks (ca. 114 g; ca. 1.5 cm thick) were surface inoculated (ca. 6.6 log CFU/g) with a rifampicin-resistant cocktail comprised of single strains from each of eight target serogroups of STEC (STEC-8; O111:H-, O45:H2, O103:H2, O104:H4, O121:H19, O145:NM, O26:H11, and O157:H7). Next, inoculated steaks were passed once through a mechanical tenderizer, and then two tenderized steaks were knitted together by passing them concomitantly through the tenderizer two additional times. Following tenderization, knitted/cubed steaks were individually cooked on a flat-surface electric skillet set at ca. 191.5°C for up to 3.5 minutes per side.

Results: The longer the cooking time, the higher the final internal temperature, and the greater the inactivation of STEC within cubed steaks. The average final internal temperature of knitted/cubed steaks cooked for 1.0 to 3.5 minutes ranged from 40.3° to 82.2°C. Cooking cubed steaks on an electric skillet set at ca. 191.5°C for 1.0, 1.5, 1.75, 2.0, 2.5, 3.0, and 3.5 minutes per side resulted in average total reductions of ca. 1.0, 2.1, 2.2, 2.8, 4.1, 5.2, and 6.3 log CFU/g in pathogen numbers, respectively.

Significance: These data validated that cooking cubed steaks on an electric skillet at 191.5°C for at least 3 minutes per side was sufficient to achieve a 5.0-log reduction of STEC-8.

P3-64 Thermal Inactivation of *Escherichia coli* O157:H7 and Non-O157:H7 Shiga Toxin-producing *E. coli* in Moisture Enhanced Non-intact Beefs as Affected by Internal Temperature, Moisture Enhancing Rate, and Resting Time by Double Pan-broiling

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Introduction: *Escherichia coli* O157:H7 (ECOH) or Non-O157 Shiga toxin-producing *E. coli* (STEC) may translocate from the meat surface to internal tissue during non-intact beef moisture enhancement (ME) process. Limited information is available regarding the thermal inactivation of Non-O157 STEC on moisture enhanced non-intact beefs.

Purpose: This study evaluated the effect of ME rate, resting time on the inactivation of ECOH or Non-O157 STEC in reconstructed non-intact beefs cooked to various internal temperatures.

Methods: Fresh 1-kg coarse-ground beef knuckles, inoculated with ECOH (2-strain cocktail) or a mixture of Non-O157-STEC O26, O45, O103, O111, O121, and O145 (5.8 ± 0.1 log CFU/g), were mixed with NaCl+Na-tripolyphosphate solutions to reach a 10 or 18% ME rate. Semi-frozen beefs were cut into 2.54-cm thick, vacuum-packaged, frozen (-20°C, 96h), and tempered (4°C, 2.5h) before double pan-broiling (Farber-ware®griller) to internal temperatures of 55, 60, 65 and 71.1°C with 0.5 or 3.5-min rest. Temperatures of the griller and beefs were monitored with thermocouples. Samples were analyzed for survivors on tryptic soy agar and sorbitol MacConkey agar plus sodium pyruvate (0.1%). Colors of the internal cooked beef were also monitored. Data (two replicates/three samples each) were analyzed using the GLM model of SPSS including independent variables and interactions.

Results: ECOH and Non-O157 STEC behave similar ($P = 0.518$) to thermal treatments. Cooked internal temperature ($P = 0.001$) of 65 and 71.1°C achieved higher reductions (4.76-5.21 log CFU/g) compared to those of 55 and 60°C (2.43-3.53 log CFU/g) regardless of ME rate and resting time. 3.5-min resting time ($P = 0.007$) or 18%-ME ($P = 0.008$) achieved an additional 0.5 log CFU/g reduction among various internal temperatures. Similar internal colors of a* (7.39), b* (17.96), L* (48.57) (10%-ME) and a* (11.36), b* (15.11), L* (46.25) (18%-ME) were observed regardless of cooked internal temperatures.

Significance: These findings will be useful by USDA-FSIS to develop risk assessments of ECOH and Non-O157 STEC on non-intact and intact beef products.

P3-65 Effect of Direct-fed Microbial Supplementation on the Presence of *Salmonella enterica* in Bovine Peripheral Lymph Nodes

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Poster

Introduction: Bovine peripheral lymph nodes (LN) contained within adipose trim, have been identified as a potential source of human exposure to *Salmonella enterica*, when incorporated into ground beef. How *Salmonella* gain entry to peripheral LN is a question yet to be answered, however recent survey data indicate that *Salmonella* prevalence and levels in feedlot environments may play a significant role. Thus there is a perceived need to identify pre-harvest measures for preventing *Salmonella* entry into this niche.

Purpose: To determine the effect of feeding a direct-fed microbial, Bovamine®†, on *Salmonella* prevalence and levels in bovine peripheral LN.

Methods: Cattle (n = 3,259) were housed in a commercial feed yard setting and divided among 24 pens (avg. 135 hd/pen), such that there were 12 treated and control paired pen replicates. Treated cattle were fed a standard ration with the addition of Bovamine® (~10^7CFU/hd/d), while control cattle were fed the standard ration. Cattle within each pen pair were harvested at the same processing plant, on the same day. At harvest, one subiliac LN was removed from 30 randomly selected carcasses per pen, for a total of 720 LN collected. All samples were analyzed for *Salmonella* prevalence and level.

Results: Average *Salmonella* prevalence for all LN tested was 41.9%, with treated found to be significantly lower than control cattle (35% vs. 48.7%; $P = 0.0189$). *Salmonella* contamination levels were generally low with the majority of LN (71.1%) containing between 0 to 20 CFU/LN. While 10.6% of LN overall were found highly contaminated (≥4.0 log CFU/LN), the percentage of treated were lower than control in this category (6.1% vs. 15%, respectively), although not significantly ($P = 0.1697$).

Significance: These data suggest that direct-fed microbials may be a beneficial component of a multi-hurdle, pre-harvest approach, aimed at decreasing *Salmonella* contamination of bovine peripheral lymph nodes.

†Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

P3-66 Determining the Validity of Two Antimicrobial Agents Applied during the Production of Further Processed Beef Products

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Developing Scientist Competition

Introduction: In 1999, the United States Department of Agriculture – Food Safety and Inspection Service declared *Escherichia coli* O157:H7 as an adulterant in non-intact beef products. In response to this declaration, many further processors of beef products are applying antimicrobial interventions. Further, all beef processors are required to address *E. coli* O157:H7 in their hazard analysis, and validate their systems to ensure identified pathogens of concern are adequately controlled.

Purpose: This project was designed to validate in-plant application of two different antimicrobial interventions (lactic acid and Beefxide) applied to multiple processing schemes (e.g., single-pass or multiple-pass tenderization and marination).

Methods: Rifampicin-resistant Biotype I *E. coli* O157:H7 surrogate microorganisms (ATCC BAA-1427, BAA-1428, and BAA-1430) were applied as a cocktail (7.8 log CFU/ml) to three beef products (boneless strip loins, top sirloin butts, and bottom sirloin flaps) prior to treatment with an antimicrobial intervention (2.5% Beefxide or 2.9% lactic acid). Following inoculation and antimicrobial spray, products were subjected to a single- or multiple-pass tenderization and/or marination process.

Results: Beefxide and lactic acid treatments resulted in statistically significant ($P < 0.05$) log reductions of surrogate microorganisms on product surfaces for all three beef subprimals. Surrogate microorganisms also were recovered from interior samples of all three beef product types after mechanical tenderization. Additionally, there were no statistical differences ($P > 0.05$) between the post-treatment surface and interior counts for the bottom sirloin flap following tenderization and marination.

Significance: These data indicate that tenderization and marination processes can transfer microorganisms into the interior of whole-muscle cuts, and suggest Beefxide and lactic acid may be similar in their efficacy as an antimicrobial applied as an intervention in the production of non-intact beef products.

P3-67 Efficacy of Various Electrolyzed Oxidizing Waters to Control *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 from Cattle Hides

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Introduction: Beef products contaminated with *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) are major causes of foodborne illness in the United States. Many previous studies reported cattle hide as major source of above mentioned pathogens. Therefore it is important to reduce levels of aforementioned pathogens it is important to reduce the bacterial contamination of the hide.

Purpose: This study was designed to determine suitability of various Electrolyzed Oxidizing water in carcass/hide wash cabinet to reduce aerobic plate counts (AC) and *Enterobacteriaceae* (EC) from uninoculated fresh cattle hides and *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 from inoculated hides.

Methods: Fresh hides were cut in to 60 by 30 cm pieces and subjected to a total of eight different treatment solutions; near neutral pH EO water (NEW- pH 6.5 at room temperature, 150 mg/l available chlorine), alkaline pH EO water (AEO- pH 11.6 at room temperature), hot alkaline pH EO water at 43°C (HAEO- pH 11.60), alkaline pH EO water spray followed by 150 mg/l available chlorine containing near neutral pH EO water spray (A- NEW- both at room temperature), Blitz™(PAA, pH 3.02 at room temperature), 5% lactic acid (LA, pH 2.04 at room temperature), deionized water (W) and no treatment (Control). All treatments were applied by spraying each treatment solution for 30s at 20 PSI, using a specially constructed hide/carcass wash cabinet.

Results: Five percent lactic acid spray treatment was found to be the most effective treatment and achieved 2.10 ± 0.56, 2.70 ± 0.30, 2.75 ± 0.42 and 2.90 ± 0.66 log CFU/cm^2 of AC, EC, *E. coli* O157:H7 and *S*. Typhimurium DT 104 reductions, respectively (limit of detection 70 CFU/100cm^2). All EO water treatments were equally effective in reducing all target microorganisms, except *E. coli* O157:H7 ($P \leq 0.05$). HAEO and A-NEW treatments yielded significant reduction of *E. coli* O157:H7 compared to other EO water treatments.

Significance: These results indicate that various EO water treatments could become viable option to control pathogens on hide during slaughter process.

P3-68 Influence of Beef Carcass Topography and Surface Composition on the Attachment of *Salmonella* and *Escherichia coli* Biotype I

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Introduction: The topography and composition of beef carcasses differ among carcass regions, and some areas of the carcass surface facilitate bacterial adhesion. The attachment properties of *Salmonella* and *E. coli* strains proposed as surrogate organisms for validation studies on beef carcasses were compared as affected by carcass tissue.

Purpose: To investigate the attachment properties of *Salmonella* and *E. coli* strains proposed as surrogates as affected by the beef carcass topography and surface composition.

Methods: Five rifampicin-resistant *Salmonella* strains and four ampicillin-resistant *E. coli* strains were inoculated on beef tissues (1.5 cm^2 on muscle, fascia, tendon, fat) and 1.0 cm^2 (bone) pieces (0.1-0.5 mm depth). The pieces were individually placed in the inoculum into polystyrene 12-well plates and stored for two different temperature conditions. Temperature conditions included 26.6 ± 2.2°C (RT) for 24h or alternating RT (30 min) -refrigeration (4.8 ± 1.3°C, 24h) -RT (30 min). After incubation time tissues were rinsed and loosely and strongly cells were enumerated on appropriate culture media and Sr value was expressed as percent of bacterial population physically attached to the surface (Sr= #loosely attached cells - #strongly attached cells /#strongly attached cells) .

Results: No differences were observed ($P \geq 0.05$) on Sr values for all beef carcass tissues (n = 60). At 26.6 ± 2.2°C, Sr values ranged from 0.47 ± 0.00 to 0.54 ± 0.02 and at alternating temperatures ranged from 0.46 ± 0.01 to 0.54 ± 0.03. No differences were observed ($P \geq 0.05$) between Sr values for *Salmonella* and *E. coli* on all beef carcass tissues and temperature conditions. The Sr values for *Salmonella* ranged from 0.52 ± 0.01and for *E. coli* from 0.52 ± 0.02.

Significance: The topography and superficial composition of tissues did not influence the attachment of bacteria. *E. coli* strains showed similar attachment properties to *Salmonella* strains confirming that they may be used as surrogates for validations studies on beef carcasses.

P3-69 Process Analysis of Cattle Slaughtering in Two Abattoirs: Influence of Process Stages on the Microbial Carcass Contamination

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Introduction: Strict adherence to good practices of slaughter hygiene, along with risk-based preventive measures, is crucial to ensure public health protection and meat quality. For the implementation of HACCP-based systems, slaughter process analysis including microbial data on carcass contamination during slaughter is required.

Purpose: The aims of this study were (i) to investigate the effects of slaughter process stages on the microbial carcass contamination, and (ii) to compare microbial loads on cattle carcasses and hides (carcass–hide ratios).

Methods: In two large-scale Swiss abattoirs, samples from 100 cattle carcasses examined at selected stages of slaughter (skinning, evisceration, trimming, washing, blast chilling) and from 100 hides and corresponding chilled carcasses were collected. Carcass and hide samples were obtained by swabbing from the neck, brisket, flank, and rump area and examined for total viable counts (TVC) and *Enterobacteriaceae*.

Results: On hides, average TVC was 5.6 log CFU cm^{-2} (abattoir A > abattoir B, difference 0.5 log CFU cm^{-2}) and *Enterobacteriaceae* were commonly detected. After skinning, average TVC on carcasses was 1.5 log CFU cm^{-2} (abattoir A > abattoir B, difference 0.3 log CFU cm^{-2}) and *Enterobacteriaceae* frequencies were <10%. After evisceration, trimming, and washing, minor changes (mainly increases) occurred. Blast chilling reduced microbial loads on carcasses from abattoir B, but reductions were limited in abattoir A (average TVC: 1.0 and 2.0 log CFU cm^{-2} in abattoir A and B, respectively). Carcass–hide ratios of the two abattoirs were comparable for TVC but differed for *Enterobacteriaceae* counts.

Significance: Average carcass contamination after skinning was low, levels slightly increased from skinned to washed carcasses, and the abattoir-specific effects of blast chilling were probably related to differences in the achieved surface desiccation. In the daily practice, carcass–hide ratios allow comparing slaughter process performance between abattoirs.

P3-70 Determining the Appropriateness of Established *Escherichia coli* Biotype I Surrogates as Predictors of Non-O157 Shiga Toxin-producing *E. coli* (STECs) Using Various Growth Characteristics

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Developing Scientist Competition

Introduction: Foodborne illness attributed to STECs has increased in the past decade, and the USDA-FSIS has declared the "Big 6" as adulterants in raw beef manufacturing. *Escherichia coli* biotype I strains were previously isolated from cattle hides and determined to be appropriate for use as surrogates for *E. coli* O157:H7.

Purpose: To determine if surrogates can be used for non-*E. coli* O157 STECs by evaluating growth characteristics.

Methods: Non-O157 Shiga toxin-producing *E. coli* (STECs) (ATCC # 2192, 2193, 2196, 2215, 2217 and 2219) and *E. coli* biotype I surrogates (ATCC # BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431) were individually cultured in tryptic soy broth at 37°C for 18h. Rifampicin-resistant surrogates were prepared and compared to the parent strains (16 bacterial strains x 3 replications). Enumeration of cultures was performed on tryptic soy agar (TSA) with incubation at 37°C for 24h. The GLM of SAS was used to identify differences in growth parameters.

Results: Growth curves of *E. coli* biotype I surrogates were similar to those of STECs throughout the evaluation, differing by 0.5 log or less at each 1-hour evaluation during the 24-hour growth period. While some statistical differences ($P < 0.05$) were detected at intervals during growth from an initial population of approximately log 3.0 to log 9.0 over 24h, trends were clearly within ranges needed for use as surrogates.

Significance: These data suggest that *E. coli* biotype I surrogates may be used to predict growth of non-O157 Shiga toxin-producing *E. coli* (STECs) within a small amount of error.

P3-71 Comparison of Predictive Models for Growth of Shiga Toxin-producing *Escherichia coli* (STEC) in Ground Beef

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Poster

Introduction: Foodborne illness caused by Shiga toxin-producing *E. coli* (STECs) has been a major concern. While predictive models for the growth of *E. coli* O157 and other *E. coli* are available, they have not been validated for growth of non-O157 STEC serogroups.

Purpose: The objective of the research was to evaluate the performance of Combase model (for *E. coli* O157:H7) and Ratkowsky square-root model recommended by Huang for the prediction of the growth of STECs in ground beef (Huang model).

Methods: Irradiated ground beef (fat, 73/27; and lean, 93/7) was inoculated with ca. 2 CFU/g of five-strain cocktails of O157:H7, O26, O45, O103, O104, O111, O121, O145, and six-serovar STEC cocktails from USDA-ARS and ATCC, respectively. Inoculated ground beef was packaged in vacuum bags, placed in programmable water baths to follow time-varying temperature profiles (sinusoidal; low or high temperature profiles) for a total of 300h and 25h, respectively. Growth data were collected and fitted into the models described by Huang and Combase. Model performance indicators (Root mean squared error, RMSE; bias factor, BF; and accuracy factor, AF) were calculated to evaluate the performance of these models.

Results: The Huang model underestimated the growth of all STECs for both temperature profiles; the average RMSE for fat and lean meat were 1.87 and 2.14 at low temperature, and 2.39 and 2.42 at high temperature profiles. Combase model over-predicted the growth of all STECs with a mean RMSE of 1.44 and 1.60 log CFU/g for fat and lean ground beef, respectively. Besides, Combase model cannot predict the growth of *E. coli* O157:H7 at temperatures below 10°C where growth of STEC can occur.

Significance: None of the two models precisely predicted the growth of STECs in ground beef. However, Combase could be a conservative option given the limited availability of predictive models for STECs.

P3-72 Prevalence of *Salmonella* spp. in Ground Beef Sold in Bogotá, Colombia

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Introduction: *Salmonella* is frequently involved in diarrheal disease throughout the world and is disseminated mainly by food, polluted waters or infected food-handlers. In Colombia, the serotypes of *Salmonella* and their prevalence in ground beef have not been characterized. Reliable detection of foodborne pathogens is critical for the protection of consumers, and new developments in rapid methods deliver higher efficiency and faster results. The 3M™ Molecular Detection Assay *Salmonella* method uses a combination of isothermal amplification and bioluminescence to rapidly detect *Salmonella* in enriched food, feed and environmental samples with high sensitivity and specificity.

Purpose: To determine the prevalence of *Salmonella* spp., in ground beef commercialized in different points of sale around Bogota, Colombia.

Methods: A total of 180 samples of ground beef were taken from 18 points of sale. 90 were obtained from two markets and 90 were obtained from two retail stores. Samples were analyzed by the 3M Molecular Detection Assay *Salmonella*. **Results** were also confirmed biochemically. Moreover, a survey was applied to determined hygienic conditions at the establishments from which samples were taken.

Results: 11.1 % of *Salmonella* spp., prevalence was found. Based on the establishment type, statistically significant differences between bacteria isolated from market and those isolated from supermarkets were established.

Significance: Greatest deficiencies on standard execution compliance were found at markets. The following risk factors resulting in product contamination were observed: lack of sanitary design in the establishments, inadequate food-handlers practices, contact with vectors, mostly insects, lack of control strategies like an adequate cold storage and handling. As consequence of good sanitary execution standards compliance at retail stores points of sale, prevalence of *Salmonella* spp. has been reduced. Use of rapid detection methods, such as the 3M™ Molecular Detection System, is a key component in a pathogen monitoring program to maintain compliance.

P3-73 Impact of Sampling Programs on the Risk of *Escherichia coli* O157 Infection from Consumption of Hamburgers Made from Australian Manufacturing Beef

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Introduction: Australia exports about 150-200,000t of manufacturing beef to the USA annually and each lot is tested for *E. coli* O157 using the robust N-60 sampling protocol.

Purpose: To evaluate the effect of different sampling programs on the risk of *E. coli* O157 infection from the consumption of hamburgers made from Australian manufacturing meat.

Methods: A total of 10,000 contaminated lots of 700×27.2kg cartons of Australian manufacturing meat were simulated in production of 100g patties for home consumption. Each simulated lot was sampled using a Binomial-Poisson-Lognormal approach, where the

- Binomial distribution gives the probability of sampling contaminated cartons;
- Poisson distribution (rate λ) gives the probability of detecting *E. coli* O157 in a carton; and
- Lognormal distribution gives variability in the average rate of *E. coli* O157 contamination (λ).

Sampling plans included N-60 (testing five samples from 12 cartons each), N-90 (testing five samples from 18 cartons each), N-120 (testing five samples from 24 cartons each) and ICMSF n=60 (testing five 50cm^2 samples from 12 cartons each). Lots in which contamination was detected were removed from the simulation and the resulting infection rate (per 100g serving) was calculated.

Results: The results indicate that compared with no sampling, which had a risk of *E. coli* O157 infection of 2.83×10^{-7}, sampling using current N-60 reduced this risk to 2.58×10^{-7}, while sampling using N-90 and N-120 had limited further impact on the risk, which were 2.53×10^{-7} and 2.51×10^{-7}, respectively. Sampling more surface area per carton (ICMSF n=60) yielded a risk of 2.44×10^{-7}. In contrast, cooking to 68°C reduced the risk to 7.30×10^{-11}.

Significance: While sampling helps detect highly contaminated lots, it does not provided effective public health protection from the consumption of undercooked burgers. Increased testing beyond the current sampling plan provides marginal additional public health benefit.

P3-74 Low Level Recovery of *Escherichia coli* O157:H7 and *Salmonella* by the BAX® System in Mechanically Collected N60 Samples of Beef Trim

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Introduction: Recognizing the consumer risk of enteric pathogens in the beef industry, microbial hazard identification strategies are expanding to include the regulation of both *E. coli* O157:H7 and *Salmonella*. Standardized sampling plans such as N60 (collecting sixty 6.25g sub-samples per production lot) are being adopted to provide fast, accurate testing for both pathogens to help reduce the risk of bacterial contamination and related illness.

Purpose: This study was designed to evaluate the DuPont™ BAX® System method for detecting both *E. coli* O157:H7 and *Salmonella* in 50% lean beef trimmings collected using the IEH N60 PLUS Sampler™, a mechanical alternative collection method developed to obtain samples more efficiently than manual excision.

Methods: Beef trimmings received from a North American beef producer were artificially co-inoculated with approximately 1 CFU/150g portion each of *E. coli* O157:H7 and *Salmonella*. Samples were combined with either 600 ml of Trypitcase Soy Broth containing 2 mg/l novobiocin (TSB+n) or propriety BAX® System MP media and incubated at 42 ± 1°C for 10-22 hours. Following enrichment, samples were analyzed by real-time PCR and confirmed according to the USDA-FSIS reference culture methods.

Results: For *E. coli* O157:H7 testing, PCR detected all 12 culture positive samples after enrichment in TSB+n for 12 hours and all 11 culture positive samples after enrichment in MP media for 15 hours. For *Salmonella* testing, PCR detected all 7 culture positive samples after enrichment in TSB+n for 12 hours and all 11 culture positive samples after enrichment in MP media for 12 hours.

Significance: This study demonstrates that beef producers can reliably use a mechanical device to collect samples and test using the BAX® System method with equivalent sensitivity to the USDA-FSIS culture methods.

P3-75 Competitive Growth between *Escherichia coli* O157:H7, *Brochothrix thermosphacta* and Other Background Microflora in Ground Beef

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Introduction: *Escherichia coli* O157:H7 is one of the major causes of contamination in meat product. Presence of *E. coli* O157:H7 may change the microbial community to compete with other background bacteria in ground meat.

Purpose: The purpose of this study was 1) to study the change in microbial community by *E. coli* O157:H7 in ground beef (GB) during storage, 2) to identify the bacteria involve in the competition and to understand mechanism of the competition.

Methods: Imported and domestic GB (13 and 37 samples, respectively) were purchased from local stores in Korea and their microbial community was compared using PCR-DGGE analysis method. Based on this information, major meat spoilage bacteria such as lactic acid bacteria, *Pseudomonas* spp., *Enterobacteriaceae* and *Brochothrix thermosphacta*, and total aerobic bacteria was monitored during storage at 5 and 10°C for 0, 1, 3, 7 and 10 days. *E. coli* O157:H7 was inoculated at the concentration of 5.11 x 10^2 log CFU/g and growth with other bacteria during storage was monitored. The result was confirmed by plate counting and PCR-DGGE analysis.

Results: PCR-DGGE analysis showed the presence of *Lactobacillus plantarum*, *Proteus mirabilis* and *Photobacterium* spp and others by contamination of *E. coli* O157:H7 while *Enterobacter* and *Citrobacter* spp were identified in the native GB. During early stage of storage, the growth of *B. thermosphacta* was significantly reduced by *E. coli* O157:H7 by 1.28 log CFU/g ($P < 0.05$). Cell free supernatant of *E. coli* O157:H7 treatment, metabolite analysis and co-inoculation with *B. thermosphacta* showed the competitive growth and presence of potential antimicrobials in *E. coli* O157:H7 to inhibit the growth of *B. thermosphacta*.

Significance: This study is the first to show the competitive interaction between *E. coli* O157:H7 and *B. thermosphacta* and to show the change of microbial ecology in GB by *E. coli* O157:H7.

P3-76 Prevalence of *Campylobacter* in Retail Ground Beef and Poultry during Spring in Lubbock, Texas

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Introduction: *Campylobacter* is the most common cause of gastroenteritis in the United States. *Campylobacter* contamination of retail ground beef and poultry poses a significant threat to human health. Monitoring this pathogen at the retail level is important to further our understanding of the efficacy of current meat and poultry interventions.

Purpose: The purpose of this study was to determine the prevalence of *Campylobacter* in retail ground beef and poultry during spring in Lubbock, Texas.

Methods: A total of 340 poultry and ground beef samples with a variety of fat:lean ratios were collected from seven stores across the city of Lubbock, Texas. Samples were rinsed and enriched with Bolton broth and processed in accordance with standard BAX protocols (Dupont Qualicon) for the detection of *Campylobacter*. A Chi-square analysis was performed using SAS 9.3 to determine relationships between samples positive for *Campylobacter* and product type.

Results: Overall, the prevalence of *Campylobacter* was 25.29% with 86 positives for all samples collected (n = 340); where 16.76% of them were from beef (n = 190) and 8.53% of were from poultry (n = 150). Beef samples alone showed a 30% positive (57) while poultry had only 19.33% positives (29). Prevalence of *Campylobacter* in ground beef was significantly higher ($P = 0.02$) than that observed in poultry.

Significance: Retail is the final step in poultry and meat processing, and it is important to monitor pathogen contamination at this level in order to verify meat and poultry interventions and the safety of the product. This research is a snapshot of *Campylobacter* contamination in ground beef and poultry products at the retail level.

P3-77 Inhibition of *Salmonella* in Feces and Soil from the Feedlot Environment Treated with *Lactobacillus acidophilus* NP51

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Introduction: *Salmonella* is ubiquitous in the cattle feeding environment and can survive long term in soil and fecal matter creating a source of contamination to the hide and gastrointestinal track of cattle during the feeding phase. When fed, *Lactobacillus acidophilus* (NP51) reduced the presence of pathogens in the digestive track of cattle. This warrants investigation on NP51 as an environmental treatment to reduce *Salmonella* in the feedlot.

Purpose: The objective of this study is to observe the effects of NP51 on fecal and soil samples inoculated with *Salmonella*.

Methods: A 1000-g of fecal and soil were collected separately from a feedlot and inoculated with a cocktail containing three strains of *Salmonella* at 10^3 CFU/g. Each sample type was divided into two 500-g aliquots. One 500-g portion was treated with NP51 at 10^9 CFU/g. The other 500-g portion for both soil and fecal material remained untreated to serve as a control. All samples were stored at 37°C and were taken at 0, 24, and 48 hours. Ten grams were diluted in 90 ml of buffer peptone water and further serially diluted and spread plated in duplicate onto XLT4 agar. This process was replicated three separate times (n = 36). Data was $\log_{10}$-transformed and analyzed using repeated measures ANOVA.

Results: There was a significant treatment effect ($P \leq 0.05$) for the fecal samples treated with NP51 with 0.4 log CFU/g less *Salmonella* detected overall compared to the untreated sample. There were no significant differences observed for soil inoculated with *Salmonella* and treated with NP51 when compared to controls.

Significance: These data indicate reductions in the presence of *Salmonella* in inoculated fecal samples treated with NP51. Decreasing *Salmonella* in the cattle feces is an important step in reducing overall prevalence in feedlot environment to help minimize the transfer of pathogens into food products.

P3-78 Controlling Attachment and Growth of *Listeria monocytogenes* in PVC Model Floor Drains Using a Peroxide Chemical, Chitosan-arginine or Heat

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Introduction: *Listeria monocytogenes* enters a poultry further processing plant with raw product and colonizes the plant as a resident in floor drains. We have shown that *L. monocytogenes* can escape floor drains, becoming airborne during wash down, creating potential for contamination of fully cooked product. Limiting growth and attachment to drain surfaces may help prevent cross contamination.

Purpose: The objective of this study was to compare a synthetic hydrogen peroxide-based chemical proven to be effective in drain sanitizing to naturally derived chitosan-arginine or heat to prevent attachment of or destroy existing *L. monocytogenes* on the inner surface of model floor drains.

Methods: *L. monocytogenes* was introduced to result in about 10^9 planktonic and attached cells within untreated PVC model drain pipes. Treatments (0.13% peroxide based sanitizer, 0.1% chitosan-arginine or 15s of hot water at 95 - 100°C) were applied immediately after inoculation, or after 24h incubation. Following treatment, all pipes were incubated for an additional 24h; planktonic and attached cells were enumerated by plate count.

Results: All treatments significantly ($P < 0.05$) lowered numbers of planktonic and attached cells recovered. Chitosan-arginine resulted in a 6 log reduction when applied prior to incubation and a 3 log reduction after the inoculum had a chance to grow. Both heat and peroxide significantly outperformed chitosan-arginine (8 to 9 log reduction) and were equally effective before and after incubation. Heat was the only treatment which eliminated planktonic *L. monocytogenes*.

Significance: Based on these data, a processor can choose between a natural solution, a chemical solution or a physical treatment to combat drain contamination. Applied at the most efficacious time, any of these techniques may lessen the potential for *L. monocytogenes* to remain as a long term resident in processing plant floor drains.

P3-79 Antibiotic Susceptibility of *Listeria* Species Isolated from Conventional and Pasture Flock Raised Poultry and Their Environments

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Introduction: *Listeria monocytogenes* has been recognized as a foodborne pathogen of public health concern in the food industry since it causes severe diseases in immuno-compromised persons and pregnant women. Due to the increasing emergence of drug resistant bacterial species, identification and investigation of antibiotic resistant *Listeria* species isolated from foods and environments should be conducted to monitor the emergence and spread of antibiotic resistance in food environments.

Purpose: The aim of this study was to evaluate the antibiotic susceptibility of *Listeria* species including *L. monocytogenes* isolated from conventional versus antibiotic-free pastured flock poultry and environments.

Methods: A total of 29 *Listeria* strains from four species (*L. monocytogenes, L. innocua, L. welshimeri* and *L. seeligeri*) isolated from conventional and pasture flock raised poultry and their environments were evaluated using 7 antibiotics (ampicillin(10 µg), ampicillin-sulbactam (10/10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), imipenem (10 µg) and tetracycline (30 µg)). These antibiotics are representatives of report group based on CLSI guideline.

Results: Most *Listeria* strains were susceptible to the antibiotics tested but 5 strains were resistant to at least one antibiotic. Two pastured poultry *L. innocua* strains isolated from soil showed resistance against ampicillin and ampicillin-sulbactam, and tetracycline, respectively. One pastured poultry *L. welshimeri* strain isolated from a chicken carcass rinsate exhibited Tetracycline resistance. Furthermore, one pastured poultry *L. monocytogenes* from grass showed ampicillin and one *L. monocytogenes* from environment exhibited ampicillin-sulbactam resistance, respectively.

Significance: Investigation of antibiotic response profiles in *Listeria* species in conventional poultry and pasture flock environments are important to monitor spreading of resistant strains to establish prevalence and origins of antibiotic resistance in food system environments.

P3-80 *Arcobacter*: Comparison of Isolation Methods, Diversity and Potential Pathogenic Factors in Commercially Retailed Chicken Breast Meat from Costa Rica

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Introduction: *Arcobacter* species have been recognized as potential food and waterborne pathogens. The lack of standardized isolation methods as well as the relatively scarce knowledge about their prevalence and distribution as emerging pathogens are due to the limitations in their detection and identification.

Purpose: This study aimed to determine the presence and the identification of *Arcobacter* in chicken breast samples commercially retailed in San José, Costa Rica, as well as to describe the adherence and invasive potential of the strains to human cells (HEp-2).

Methods: Fifty chicken breast samples were collected from retail markets in the metropolitan area of the country. Six different isolation methodologies were applied for the isolation of *Arcobacter*. Isolation strategies consisted of combinations of enrichments in de Boer or Houf selective broths and subsequent isolation in blood agar (directly or with a previous passive membrane filtration step) or *Arcobacter* selective agar. Suspicious colonies were identified with a genus-specific PCR, whereas species-level identification was achieved with a multiplex-PCR.

Results: The overall isolation frequency of *Arcobacter* was 56%. From the isolation strategies, the combination of enrichment in Houf selective broth followed by filtration on blood agar showed the best performance, with a sensitivity of 89% and a specificity of 84%. A total of 46 isolates were confirmed as *Arcobacter* with the genus-specific PCR, from which 27 (59%) corresponded to *A. butzleri*, 9 (19%) to *A. cryaerophilus* and 10 (22%) were not identified, with this multiplex PCR. Regarding the potential pathogenicity, 75% of the isolates presented adherence to HEp-2 cells, while only 22% were invasive to that cell line. All the invasive strains were *A. butzleri* or non-identified strains.

Significance: The results evidence the presence of potentially pathogen *Arcobacter* in poultry and highlight the recognition it should receive from public health authorities.

P3-81 Incidence of *Listeria* spp. and *Listeria monocytogenes* in Broilers at Abattoir in Algeria

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Introduction: The involvement of *Listeria monocytogenes* as an infecting agent, in both sporadic and outbreaks listeriosis cases, has been related to the consumption of different food stuffs of vegetal and animal origins. Poultry products have not been directly implicated as sources of outbreaks, while ready-cooked chicken and under-cooked chicken have been linked to sporadic cases of human listeriosis.

Purpose: Objectives of this study were an estimation of *Listeria monocytogenes* prevalence in poultry carcasses at broiler abattoirs and their molecular characterization by Multiplex-PCR and the PFGE method.

Methods: Prevalence of *Listeria* contamination in three industrial poultry abattoirs was investigated by sampling carcasses at the end of processing, after packaging and refrigerating. A total of 212 carcasses were collected: 52 from abattoir A and 80 from both abattoir B and C. *Listeria* screening was performed according to ISO 11290-1 method.

Results: 46.7% of samples presented *Listeria*: *L. monocytogenes* (8.9%), *L. innocua* (32.5%), *L. grayi* (4.7%) and *L. welshimeri* (0.5%). The *Listeria monocytogenes* contamination rate varied from 5 to 11.5 %, between the three abattoirs. *Listeria innocua was* the most commonly identified species: 8.8% in abattoir A, 33.7% in both abattoirs B and C. 26 isolates were subjected to characterization by serogrouping by Multiplex –PCR. *L. monocytogenes* serogroups were IIa , IIb and IVb. IIa was common to all abattoirs; IIb and IVb were reported only in abattoir C. The serotypes (1/2a, 1/2b, and 4b) cause the vast majority of clinical cases. Serotype 1/2a is the most frequently isolated from food, serotype 4b causes the majority of human epidemics.

Significance: The study demonstrated the high prevalence of *Listeria* spp. and specifically *L. monocytogenes* in raw broiler, this high incidence is a problem that should be of concern to the food producers. Sanitation programs to prevent or at least to reduce *Listeria* contamination should be adopted.

P3-82 Optimizing Air-chilling of Poultry Carcasses Using Aided Airflow in Carcass Cavity

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Developing Scientist Competition

Introduction: Rapid cooling of poultry carcasses immediately after slaughter is critical to assure their safety. Previous studies have shown that the carcass surface in the cavity exhibits lower cooling rates and consequently, a higher risk of pathogen growth if present. Improving air circulation inside carcass cavity may help improve chilling rates and food safety of poultry carcasses.

Purpose: To study cooling rates and its impact on microbiological safety of poultry carcasses as affected by aided airflow in carcass internal cavity.

Methods: Two broilers were placed in a pilot air chiller: one used as a control, and the other one connected to a prototype device to aid airflow inside the carcass cavity. The experiment was repeated six times using 0.3, 0.5, 0.7, 1.0, 1.3, and 2.1 m/s inlet airflows to the carcass cavity. Temperatures at eight different locations within each carcass, and operating parameters of the chiller (air velocity, air relative humidity, and air temperature) were collected at 10 s intervals during air-chilling. Experimental data in addition to data obtained from a validated computer model for simulating air-chilling of poultry carcasses were used to study the effect of the aided cavity airflow on chilling rates and estimated growth of *Salmonella* spp.

Results: Aided airflow in carcass cavity resulted in 20-34% reduction in carcass chilling time (1.2 ± 0.3 h), and 19-72% reduction in estimated growth of *Salmonella* spp. (0.1 ± 0.04 log CFU/ml). Optimal inlet airflow ranged between 0.3-0.8 m/s depending on carcass size and chiller operating conditions; resulting in less than 30% increase in moisture loss. Higher inlet airflows resulted in significant moisture loss (up to 85% increase), and did not provide additional reductions in chilling times.

Significance: Increasing the airflow inside carcass cavity is a potential method to improve air-chilling rates and microbiological safety of poultry carcasses. Optimal operating parameters for air-chilling of poultry carcasses can be estimated with a computer simulation model available to use at http://foodsafety.unl.edu.

P3-83 Surveillance and Antimicrobial Resistance of *Salmonella* and *Campylobacter* in Poultry Products in Farmer, Organic and Conventional Markets

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Introduction: *Salmonella* and *Campylobacter* are among the most frequently reported causative agents of foodborne illness in the US. They are often associated with poultry meat and other poultry products. Locally grown free-range pasture poultry and poultry products are mostly sold in farmer markets without any professional microbiological quality control. Therefore, it is important to understand the prevalence of these bacterial pathogens in poultry products in markets.

Purpose: Determination of the prevalence and antimicrobial resistance pattern of *Salmonella* and *Campylobacter* in poultry products in farmer and organic markets and comparison with the products available in conventional markets.

Methods: A total of 337 samples were collected and analyzed. Each of the samples was mixed vigorously at 1:9 (wt/vol) with buffered peptone water. Following enrichment in Luria-Bertani broth (for *Salmonella*) or Bolton Broth (for *Campylobacter*) supplemented both with 5% sheep blood, cultures were streaked out on Karmali or XLT-4 agar for the isolation of *Salmonella* and *Campylobacter*, respectively. Presumptive colonies were confirmed by biochemically and PCR analysis. Antimicrobial resistance was tested using the agar dilution method and χ^2 tests were used for statistical analysis.

Results: Prevalence of *Salmonella* in poultry products from farmer, organic and conventional markets were significantly different ($P = 0.0004$), with rates of 6.7%, 35.9% and 17.9%, respectively. However, the antibiotic resistance of isolated *Salmonella* from these markets was not significantly different. For *Campylobacter*, their prevalence in poultry products from these three markets were significantly different ($P = 0.029$), with rates of 33.3%, 14.1%, and 28%, respectively. In addition, the antibiotic resistance of isolated *Campylobacter* from these markets was also significantly different ($P < 0.0001$) with rates of 10%, 18.2%, and 85.9%, respectively.

Significance: The results show higher prevalence of *Salmonella* in the product collected from organic markets whereas *Campylobacter* in farmer markets. *Campylobacter* isolates from conventional markets are comparatively more resistant to antibiotic.

Poster

P3-84 Presence of *Listeria* spp. and *Listeria monocytogenes* in Environments and Products in a Plant Processing Chicken Meat

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Introduction: The presence of *L. monocytogenes* in products ready to eat must be well controlled. This control must be based on the environmental and in products bacterium detection. *Listeria* spp results as an alert indicator to minimize the risk of *L. monocytogenes.*

Purpose: The aim of this study was to assess the presence of *Listeria* spp and *L. monocytogenes* in environments and products coming from areas of low risk (LR) and high risk (HR) of a plant producing poultry meat ready to eat.

Methods: In the areas of the LR and HR environments, 26 and 411 samples, respectively, were collected with a sponge. In products 55 samples were collected before cooking (LR) as well as 62 samples after cooking (HR). All samples were analyzed by VIDAS® LDUO (bioMérieux). The colonies of *Listeria* were identified by the VITEK 2™system (bioMérieux).

Results: In LR environment *L. innocua* and *L. monocytogenes* were isolated in 19.3% and 69.2% of the samples, respectively. In HR, *L. innocua* was isolated in 3.6% of samples analyzed, *L. welshimeri* 0.5% and *L. monocytogenes* in 1.9%. In products before cooking, *L. innocua* was found in 14.5%, *L. welshimeri* 3.6% and *L. monocytogenes* in 76.3%. After cooking, *L. innocua* was isolated in 1.6% of product samples and *L. monocytogenes* in 4.8%. *L. welshimeri* was not found in the products.

Significance: Proper cooking allowed the control of *Listeria* spp as well as in processed chicken meat products, but there is a risk of contamination of the product after this step, possibly due to contact with contaminated surfaces. Control of *Listeria* spp and *L. monocytogenes* must be a set of actions of production practices and environmental control and in the end product.

P3-85 Genetic Diversity of *Listeria monocytogenes* Isolated in Environments and Products of a Meat Processing Plant

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Introduction: The genetic diversity of *L. monocytogenes* in food production environments may represent different input sources of environmental contamination, increasing the challenge for the control of the bacteria in the end products.

Purpose: The aim of this study was to assess the molecular diversity of *L. monocytogenes* isolated from environments and ready to eat of a poultry meat processing plant.

Methods: Seventy isolates of *L. monocytogenes* obtained from environments (26) and products (44) coming from the low risk areas (LR), before cooking and high risk (HR) after cooking were assessed. The isolates were analyzed by DIVERSILAB ® (bioMérieux) to compare the genetic profile.

Results: Of the 70 isolates, 18 were from the LR environment and resulted in 10 distinct genetic profiles. The eight isolated from HR environment resulted in four different profiles. In products, the 41 isolates coming from samples analyzed before cooking resulted in the formation of fifteen distinct profiles and the three isolates from samples generated after cooking, two different profiles. No similar genetic profiles were found of *L. monocytogenes* isolated from the environment and the ones isolated from product, nor between those found in the LR area against those obtained in the HR area.

Significance: The results show that despite *L. monocytogenes* having been found in products and in the environment of the high risk area there is no passing of bacteria from the LR area to the HR, showing the effectiveness of sanitary barrier as well the cooking process.

P3-86 Thermal Death Time Model for *Salmonella* in Ground Chicken Supplemented with Commercial Olive and Pomegranate Extracts

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Introduction: The application of heat is a widely used effective strategy for destroying pathogens in ground meat.

Purpose: Since inadequate cooking time and temperature are significant factors that may result in foodborne illness, this study was conducted to quantify the interactive effects of olive and pomegranate extracts (each at 0.4 – 2% w/w) on the heat resistance of a mixture of eight-strain *Salmonella* serotypes in ground chicken.

Methods: The inoculated meat samples were heated in a water bath at 55, 56.5, 58.7, 61 and 62.5°C for various times that were predetermined based on the heating temperature. After heating, the surviving microbial population was recovered on tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate solution. A survival model was then fitted to the data using a curve fitting program and decimal reduction times (D-values) were calculated. The D-values were transformed to the natural logarithms and mathematically analyzed by second order response surface regression to develop a regression model for temperature, olive and pomegranate extract, each of which may contribute to the inactivation of the pathogen.

Results: The D-values at 56.5ºC in unsupplemented ground chicken of 24.3 min was reduced to 11.3 min in ground chicken with added 1% olive and 1% pomegranate extract. While supplementing chicken with either extract increased sensitivity of the pathogen to heat, the simultaneous addition of both extracts exhibited a synergistic effect, as evidenced by a 53.5% reduction of the heat resistance. These results suggest that the described thermal death time predictive model can be used to estimate D-values in ground chicken for any combinations of temperature and food-compatible olive and pomegranate powders.

Significance: The facilitated heat reduction has the potential to enhance the microbial safety of cooked chicken products.

P3-87 Withdrawn

P3-88 Recovery of Shiga Toxin-producing *Escherichia coli* in Tenderized Veal Cordon Bleu Following Cooking on an Electric Skillet

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Introduction: The implication of veal products in several recalls due to contamination with Shiga toxin-producing *Escherichia coli* (STEC) and USDA FSIS verification sampling results revealing a higher percent positive rate of STEC in veal than in beef products provides justification for validating cooking practices for preparing veal products, particularly those processed via mechanical tenderization.

Purpose: Evaluate the viability of STEC in tenderized cordon bleu veal cooked on a flat-surface electric skillet.

Methods: Veal cutlets (ca. 75 g; ca. 0.34 cm thick) were surface inoculated with ca. 6.6 log CFU/g of a rifampicin-resistant, eight-strain cocktail of STEC (STEC-8; O111:H-, O45:H2, O103:H2, O104:H4, O121:H19, O145:NM, O26:H11, and O157:H7). Following inoculation, cutlets were mechanically tenderized via single passage through a "Sir Steak" tenderizer. Next, cordon bleu cutlets were prepared by laying one slice of ham and one slice of Swiss cheese between two tenderized cutlets, and then coating the cutlets with pre-sifted flour, liquid whole eggs, and flavored bread crumbs. For each of three trials, three inoculated and tenderized cutlets were individually cooked on a pre-heated skillet (ca. 191.5°C) for 4 to 10 min per side.

Results: Cooking tenderized veal cordon bleu on a pre-heated skillet for 4, 5, or 6 min reduced STEC levels by ca. 1.2, 2.5, and 3.0 log CFU/g, respectively. However, cooking cutlets for 7, 8, 9, or 10 min resulted in reductions of ca. 6.0 to 6.2 log CFU/g.

Significance: These data validate that cooking tenderized veal cordon bleu for at least 7 min per side on an electric skillet set at ca. 191.5°C is sufficient to achieve a ≥5-log reduction of STEC and, in turn, to appreciably lower the likelihood of illness if cutlets are contaminated with this pathogen.

P3-89 Prevalence of *Listeria monocytogenes* and *Listeria* spp. in Raw Meat and Environmental Samples at Retail in Istanbul, Turkey, and Their Antibiotic Resistance

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Introduction: *Listeria monocytogenes* is a foodborne pathogen and possibly causes listeriosis in humans.

Purpose: In this study, a total number of 550 raw meat and 300 environmental samples were investigated for the presence of *Listeria monocytogenes* and other *Listeria* spp. in Istanbul, Turkey.

Methods: The meat samples were analyzed for the presence of *Listeria* spp. using the enrichment, isolation and identification methods recommended by Food and Drug Administration. Two hundred fifty raw minced meat samples, 100 red meat samples, 100 raw meatball samples, and 100 chicken piece samples were obtained from the retail markets. The environmental samples such as water drainage and equipment's surface were collected from the retail markets by sampling or using commercial Hygislide (RTA Laboratories) kits. All samples were collected aseptically and stored at 4°C until microbiological analysis. Antibiotics susceptibility of the *L. monocytogenes* isolates were tested by the disc diffusion method. The strains of *Listeria* spp. were also identified by the API *Listeria* (BioMerieux) commercial test kits.

Results: *Listeria monocytogenes* (22.3%), *L. innocua* (52.4%), and *L. welshimeri* (9.3%) species were isolated predominantly from raw meat samples and *L. monocytogenes* (22.5%) and *L. innocua* (33.8%) were also found in environmental samples. Although the *Listeria monocytogenes* isolates from the meat and environmental samples were usually susceptible to most antibiotics, there were some resistant strains against tested antibiotics such as gentamicin, vancomycin, erythromycin, tetracycline, and ampicillin, etc., in raw meat and environmental samples at retail markets in Istanbul, Turkey.

Significance: These findings indicate the prevalence of *L. monocytogenes* in raw meat and environmental samples as well as antibiotic resistance characteristics of these isolates in Istanbul, Turkey.

P3-90 Monitoring of Foodborne Bacteria from Meats and Environmental Factors of HACCP Applied Retail Shops in Korea

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Introduction: HACCP is an internationally recognized system for food safety management. Particularly, this system is required to meat processing plants because livestock products are vulnerable to microbial contamination during the processing process. It is important to monitor the hygiene level continuously as well.

Purpose: This study is to report about the monitoring results of hygiene levels and microbiological hazard analysis of HACCP applied meat retail shops. Moreover, we analyzed the correlations with the foodborne bacteria and environmental factors.

Methods: A total of 113 meats and 340 environmental samples were collected from 60 HACCP applied retail shops located in geographically different areas in 2012. The isolation was performed for the indicator organisms (aerobic bacteria*, E. coli* and coliform), *S. aureus*, *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7. To characterize the pathogens isolated, PCR including Repetitive-Element PCR was conducted for virulence genes and molecular typing. The results were analyzed by ANOVA and DiversiLab profile system.

Results: The total bacteria count was 4.50 ± 1.10 log CFU/g (beef) and 4.77 ± 0.99 log CFU/g (pork), and was more found from the meat of large-scale shops than small ones despite no significant difference. Besides, 13 foodborne pathogens we isolated, 9 isolates were frequently found in large-scale shops (9/13, 69.2%). Of the all pathogens, 9 *L. monocytogenes* carrying *inlA*, *inlC*, *inlJ*, *prs*(7/7, 100%) and *actA*(6/7, 85.7%) were belong to 1/2a, 1/2c, 4b type, and the others were 4 *S. aureus* which were not detected any toxin. The Rep-PCR results showed genetically high relatedness (>90%) for except for each 1 isolate.

Significance: This study suggests that the large-scale meat shop could be more contaminated than the small/middle shops because of large amount of livestock products although HACCP was applied. Additionally the presence and generically high similarity of foodborne pathogenic bacteria implies the possibility of same channel for contaminated raw meat.

P3-91 Microbiological Hazards in Veal Slaughter: Identification of Contributing Factors

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Introduction: Food Safety and Inspection Service (FSIS) began distinguishing a sample from raw beef from a sample from veal in January 2011. FSIS test results from January 2011 through December 2013 show an increased percent positive rate for STEC in trimmings produced from veal as compared to trimmings produced from other cattle slaughter classes.

Purpose: Determine risk factors in veal that contribute to the increased percent positive for STEC.

Methods: FSIS conducted a review of Food Safety Assessments (FSAs), noncompliance records (NRs), and onsite visits to twelve (12) veal slaughter establishments with a history of STEC positive results during January 2011 through December 2013 to identify concerns unique to veal slaughter.

Results: FSIS identified common deficiencies contributing to the higher percent positive rate for STEC in veal: sanitary dressing deficiencies, ineffective antimicrobial intervention implementation, and inappropriate use of microbial data in decision-making.

Significance: The results suggest that deficiencies concerning the slaughter process and use of microbial data contributed to the higher percent positive for STEC in veal. More research is needed to determine if there are additional risk factors that contribute to the higher percent positive for STEC in veal.

P3-92 The Use of Liquid CO_2 as a Conveying and Dispersing Agent to Simultaneously Chill Meat Products and Broadcast Various Antimicrobial Processing Aids during a Meat Mixing Process

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Introduction: The $CO2^+$ process is designed & developed by Air Liquide to deliver a small volume of biocide (less than .0025 gallons per pound of meat) into LCO_2 & evenly distribute it throughout the entire mass of meat during the standard liquid CO2 injection cycle.

Purpose: The purpose of this study was to evaluate effectiveness of commercially available biocides in $CO2^+$ process agents in lab scale & full-scale process.

Methods: Initial tests were done in an industrial scale $CO2^+$ platform (1000 lb meat mixer) to validate uniform distribution of biocides. Tests were done with fluorescent dye and buffered acidic solution. Samples were taken from multiple locations & analyzed mixing consistency. Commercially available biocides were tested in 3 groups which represent the industrial application of CO2 chilling technology. The biocides were tested against *E. coli* on ground beef & beef trim and against *Salmonella* on turkey & chicken trim. Initial screening work was done at Air Liquide and validation studies were conducted at USDA. Selected few antimicrobial agents were tested at commercial scale (1000 lb meat/batch) CO2+ platform.

Results: Commercial scale tests confirmed the ability of this process to uniformly distribute small amounts of antimicrobial agents in meat. The results confirmed by dye test, and pH reduction in meat (from pH ~ 5.8 to <4 depending on dosage of antimicrobial agent). In general, commercial scale tests (non-inoculated, 1000 lb batch) proved that it is possible to reduce aerobic plate count up to 3 logs CFU/g , and greater than 50% reduction of *Salmonella*. Inoculated lab tests, result showed reduction of ≤ 3.00±xx log CFU/g for both *Salmonella* & *E. coli*.

Significance: The benefit of this process to the processor is to provide a last point of intervention where harmful bacteria can be reduced.

P3-93 A Field Study on the Evaluation of Hygiene and Safety in a Meat Industry Using Classical Microbiological and Typing Methods

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Developing Scientist Competition

Introduction: A systematic approach that combines classical microbiological analyses and molecular characterization for monitoring the hygiene and product safety of a meat processing plant may assist in the improvement of hygiene and disinfection practices.

Purpose: (i) To evaluate the hygienic level and (ii) to monitor the prevalence and spread of *L. monocytogenes* and *Salmonella* spp. in the plant environment and the final products of a meat industry over a 3 years period.

Methods: Environmental, raw material, and final product samples were collected during the past 3 years from a Greek meat processing industry with exports potential. Total viable counts (TVC), *Escherichia coli,* and total coliforms were enumerated on all samples. The presence of *L. monocytogenes* and *Salmonella* spp. was determined according to ISO 11290-1 and 6579:2002. Isolates of the two pathogens were serotyped using multiplex-PCR. Genotypic subtyping by PFGE was used to identify potential routes of cross-contamination and persistence.

Results: In total 2541 samples were analyzed in the period 2011-2013. The mean population of TVC, coliforms, and *Escherichia coli* was 5.6, 3.5, and 1.2 log CFU/g, respectively. *Salmonella*'s prevalence was 5.3% (134 positive samples) with 41.1% being *S.* Infantis. A total of 183 (7.2%) samples were found positive for *L. monocytogenes.* Of these 65.6% belonged to 1/2a, 25.2% to 1/2c, 3.9% to 1/2b and 5.5% to 4b serogroup. Utilization of molecular tools revealed several cross-contamination routes as indicated by the identification of same *Salmonella* and *Listeria* strains in different final products sharing same processing steps. Furthermore, the isolation of identical strains in different production lines and on personnel hands revealed straightforward cross-contamination scenarios. Finally, insufficient sanitizing procedures may favor the persistence of the pathogens as manifested by systematic isolation of certain strains within 8 months period.

Significance: Results may be used by the industry to i) evaluate hygiene of raw materials and suppliers and ii) reassess the hygiene practices of the personnel and the regular disinfection program, in order to improve the quality and safety of the final products. This work has been supported by the project "Efficacy of NOVEL analytical techniques to prEdict the quality and safetY of newly developed pErishable food products 11SYN_2_1528" co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the O.P. "Competitiveness and Entrepreneurship (OPC II)" ROP Macedonia – Thrace, ROP Crete and Aegean Islands, ROP Thessaly – Mainland Greece – Epirus, ROP Attica, Framework NSRF 2007-2013, COOPERATION 2011.

P3-94 Consumer Behaviors and Motivations for Purchasing Meat and Poultry Raised without the Use of Antibiotics

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Introduction: Antibiotic-resistant foodborne pathogens put consumers at greater risk of drug treatment failure and more virulent infections than those pan-susceptible. However, it is unknown if minimizing the likelihood of contracting an antibiotic-resistant infection is likely to drive consumer purchasing behavior.

Purpose: What influences, determines, and drives decisions of shoppers that self-report purchasing meat and poultry raised without the use of antibiotics? Prior studies have been conducted to elucidate understanding of consumer purchasing, but no study has specifically looked at whether and how preventing antibiotic resistance emergence is a possible motivator in meat and poultry purchasing decisions.

Methods: Using a systematic qualitative study approach, of grounded theory in research design, embedded in the social constructivist paradigm, this research uncovers decision-process and motivation phenomenon that consumers have when specifically buying meat and poultry raised without antibiotics. Social media was used to recruit individuals (n=148) for recorded phone interviews. Random sampling of respondents and saturation in identifying themes, interviews of consumers that already engage in this behavior were used in data analysis. QSR NVivo 10 software was used to uncover themes in purchasing phenomenon.

Results: Purchasing decisions by this subset of consumers go beyond utilitarian and incorporate hedonic, altruistic, and trust as motivators in behavior adoption. A temporality of behavior change starts with awareness, which then incorporates perceptions and motivations, and finally leads to permanent behavior adoption. Uncovering adoption phenomenon, three themes emerged. First, each consumer experienced a "tipping point." A second phenomenon identifiable was an element of altruism. And, third all consumers held "trust" in the products that they were purchasing.

Significance: Meat and poultry producers may believe that food safety, convenience, economic motivators and labeling are all primary influences in a consumers purchasing decision. This study illuminates how awareness, perception, and motivation to public health factors, previously unexamined, lend permanent shifts in purchasing behaviors.

P3-95 Presence of *Clostridium difficile* in Retail Meats and Farm Environment

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Introduction: New foodborne pathogens continue to emerge, re-emerge and persist. Globalization of food supply elevates the exposure of individuals to new disease agents. *Clostridium difficile* has been isolated from various foods and there are increasing concerns that it might be a foodborne and zoonotic pathogen.

Purpose: The objective of this study was to evaluate the presence of *C. difficile* in raw meats and farm samples including soil, feathers, and animal feces.

Methods: Samples from chicken meat (n = 77), turkey meat (n = 62), pork meat (n = 52), feces (n = 36), feathers (n = 36), and soil (n= 36) were collected and analyzed for *C. difficile.* Test samples were prepared and plated on medium Cycloserine-Cefoxitin-Fructose Agar (CCFA) and Blood Agar plates. All plates were subjected to anaerobic conditions at 37°C for 48h. Colonies were further tested for Indole and L-Proline aminopeptidase production by using *C.diff* PRO™ kit. To confirm *C. difficile* isolates, DNA was isolated using the phenol-chloroform method. *C. difficile* was confirmed using the polymerase chain reaction amplification of the toxin A and B genes (tcdA and tcdB).

Results: Presumptive colonies showed a yellowish-green appearance when viewed under UV light with a characteristic cresol odor. Some of the colonies tested positive for Indole and L-Proline aminopeptidase production. The prevalence of *C. difficile* in pork, chicken, and turkey was 6.8%, 4.2%, and 3.7%, respectively. Presence of *C. difficile* was also indicated in farm samples feces (13%), feathers 2.8%, and soils 5.5%.

Significance: The results of this study suggest that the prevalence of *C. difficile* in the U.S. retail meats and farm environment is low. Nevertheless, the presence of *C. difficile* in retail meat indicates its potential as an emerging foodborne pathogen.

P3-96 Impact of Natural Nitrite and Cherry Powder on the Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Cured Turkey Breast According to FSIS Appendix B

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Developing Scientist Competition

Introduction: A previous study confirmed that 100 ppm natural or purified nitrite provided equivalent inhibition of *Clostridium perfringens* outgrowth during 15h extended cooling according to USDA, FSIS Appendix B; furthermore, 547 ppm ascorbate, regardless of source, enhanced the antimicrobial effect of nitrite. However, typical levels of nitrite and ascorbate found in alternatively cured products are lower than those regulated for the extended cooling option of Appendix B.

Purpose: Compare outgrowth of *Clostridium perfringens* in turkey breast cured with various levels of nitrite from cultured celery juice powder and ascorbate from cherry powder during 15h cooling.

Methods: Ten treatments of deli-style turkey breast (76% moisture, pH 6.2, 1.2% NaCl) were prepared based on a two-factor full factorial design comprised of nitrite from celery powder (50, 75, and 100 ppm) and ascorbate from cherry powder (0, 250, and 500 ppm) and an uncured control. Treatments were inoculated with *C. perfringens* spores (three-strain mixture) to yield 3 log CFU/g. Individual 50-g portions were vacuum-packaged, cooked to 71.1°C and cooled from 54.4°C to 26.7°C in 5 hours and 26.7°C to 7.2°C in 10 additional hours. Triplicate samples were assayed for growth of *C. perfringens* at 0, 5, 10, and 15h by plating on tryptose-sulfite-cycloserine agar; experiments were replicated three times.

Results: The control, 50, 75, and 100 ppm nitrite and 50 ppm nitrite + 250 ppm ascorbate supported a 4.5 ± 0.4, 3.9 ± 0.2, 3.5 ± 0.3, 2.2 ± 0.6, and 1.5 ± 0.9 log increase, respectively, at 15h. In contrast, <1 log growth was observed through 15h in the remaining treatments.

Significance: This study confirmed that ascorbate enhances the antimicrobial impact of nitrite against *C. perfringens* at levels representative of alternatively cured meats, and demonstrated that products containing at least 75 ppm nitrite and 250 ppm cherry powder can be safely cooled over 15h according to Appendix B.

P3-97 Impact of pH and Water Activity on Growth of *Staphylococcus aureus* in Shelf Stable Ready-to-Eat Snack Sausages

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Introduction: *Staphylococcus aureus* is a pathogen of concern in shelf stable meats because it can grow in low pH and water activity (a_w) environments. Historically, USDA has accepted shelf stable meat and poultry as having a a_w <0.91, pH <5.0, or a combination of a_w <0.95 and pH <5.2. Recently, the validity of these critical parameters has come into question.

Purpose: Investigate the impact of pH and a_w on the inhibition of *Staphylococcus aureus* in shelf stable snack sausages acidified with citric acid.

Methods: Three batches of cured beef-pork snack sausages were prepared using encapsulated citric acid to achieve pH values of 5.6, 5.1, or 4.7, cooked to 71°C, and then dried to a_w 0.96, 0.92, or 0.88 to yield nine treatments. Treatments were surface-inoculated with 3-log CFU/g *S. aureus* (three-strain mixture) and individual 25-g portions were vacuum-packaged and stored at 21°C for 4 weeks. Triplicate samples were assayed for *S. aureus* growth at 0, 1, 2, and 4 weeks by plating on Baird-Parker agar with egg yolk tellurite enrichment; experiments were replicated twice.

Results: The treatment with a_w of 0.96 and pH of 5.6 supported 5.5 and 7.0 log *S. aureus* growth at 1 and 4 weeks, respectively. In addition, *S. aureus* populations increased ~1-log within 1 week for treatments with pH of 5.6 and either a_w of 0.92 or 0.88 but no additional growth was observed for the rest of the study, thus not confirming shelf stability. In contrast, none of the treatments with pH ≤5.1 and a_w <0.96 supported *S. aureus* growth throughout this storage period.

Poster

Significance: This study provided new pH and a_w parameters, similar to those previously outlined by USDA, that effectively inhibit *S. aureus* growth in semi-dry, pH-reduced snack sausages. Results also suggest that reduced pH has greater relative impact on *S. aureus* growth than a_w in shelf-stable snack sausages.

P3-98 Reduction of *Listeria monocytogenes* on Beef Franks Utilizing Targeted Directional Microwave Technology

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Introduction: *Listeria monocytogenes* is one of the leading causes of death in the U.S. among foodborne bacterial pathogens with infections linked to consumption of contaminated food, raw and even post-packaged meat products. Targeted directional microwave (TDM) technology is a novel non-traditional sterilization method that uses microwave frequencies to kill microorganisms in a short amount of time and is proven effectively in inactivation of *Salmonella* in shell eggs and inhibition of mold in white enriched bread.

Purpose: The objective of this research is to determine the ability of the TDM to reduce *L. monocytogenes* on inoculated beef franks in package.

Methods: Beef franks were surface-inoculated with four-strain *L. monocytogenes* at 10^3 CFU/g and cooled down in cold temperature storage (4°C). In duplicate, the samples in packages were subjected to seven treatments using the TDM to vary levels of microwave power and microwave application time while monitoring product temperature change. After treatment, serial dilutions were performed and plated on modified oxford agar followed by incubation at 35°C for 24 hours.

Results: When compared to the control sample, there were significant reductions ($P < 0.05$) of *L. monocytogenes* on beef franks in packages after treatments with varying TDM parameters controlling energy, exposure time and temperature. Four out of seven treatments had reductions of 1.35 to 2.86 log CFU/g. Only samples from one of the treatments had an internal temperature above the thermal kill temperature (71.1°C) of *L. monocytogenes*.

Significance: The data from this experiment suggest that TDM technology is an effective intervention against *L. monocytogenes* on beef franks in packages providing 1 to just less than 3 log CFU/g reduction.

P3-99 A Method Comparison of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate Method for the Enumeration of Yeast and Mold

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Introduction: Yeasts and molds can grow in a wide range of conditions making them difficult to control. These organisms have become critical indicators in monitoring the quality of food products but the 5-7 day incubation time required by the traditional cultural methods can be quite burdensome. The 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate (RYM) was developed with a new indicator technology that makes colonies easier to interpret and can produce results within 48-60 hours.

Purpose: The purpose of this independent evaluation was to compare the new method to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 method for a broad range of foods as part of the AOAC Research Institute™ validation process.

Methods: A total of 10 matrices were evaluated: yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties. Naturally contaminated samples that covered three levels of contamination (low level 10 -100 CFU/g, medium level 100-1,000 CFU/g, and a high level 1,000-10,000 CFU/g) were analyzed. Those not naturally contaminated were artificially contaminated with a yeast or mold, and 5 replicates were analyzed at 4 levels (uninoculated, low, medium, and high).

Results: A paired *t*-test was conducted at $P = 0.95$ for each matrix. No significant difference was observed between methods in 4 out of 10 matrices (yogurt, apples, ground beef and sausage.) For 5 of the matrices, sour cream, sandwiches, ready-made pie, almonds, and frozen bread dough, a statistically significant difference was observed for the high level of detection with the new method. No significant statistical difference was observed among the new method and both of the reference methods for the low and medium levels for each of the five matrices. For dehydrated soup, the new method recovered 3 separate levels of the target organism, but no recovery of the target analyte was detected by the reference methods.

Significance: The new Rapid Yeast and Mold Count Plate Method demonstrates reliability as a rapid alternative for the enumeration of yeast and mold in as little as 48 hours.

P3-100 Determination of *Alternaria* Growth and Mycotoxin Boundaries in Tomato Puree

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Introduction: *Alternaria* species were reported to be the most common fungi affecting either tomato fruit and plant, causing the so called black mould of tomato. Rapid infection of *Alternaria* in tomato may occur on the crop or post-harvest yielding high economical loss due to spoilage of industrialized products such as tomato purée. Under optimal growth conditions, *Alternaria* spp. may also produce various mycotoxins. Alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) are mycotoxins commonly found in tomatoes and tomato products, representing a serious risk for human health related to the consumption of these products.

Purpose: This study aims at defining boundaries for growth and mycotoxin production in order to optimize product formulation and shelf life.

Methods: A toxigenic isolate of *Alternaria alternate* (ITEM8176) isolated from tomato fruit affected by black mould and deposited at the ISPA collection, Italy (ITEM accession: http://www.ispa.cnr.it/Collection/) was used for growth and mycotoxin production assessment. Growth ability of the strain was determined after inoculating fungal ascospores (7 day-old culture) on cold break tomato purée supplemented with agar and followed by regular fungal development observations. A total of 6 levels of pH and 10 levels of temperature were tested, for 3 replicates, to define pH and temperature boundaries where fungal development and mycotoxin production occurred. The pH of tomato purée based medium was fixed at 2, 3, 4, 5, 6 and 7 while plates incubation was performed at 6.5, 10, 15, 20, 25, 30, 35 and 37°C. Analysis of mycotoxins (AOH, AME and TeA) was performed by HPLC with UV/DAD detection according to an adapted protocol.

Results: Stability of pH and water activity of tomato purée based media was checked throughout the experiments. Growth was observed above a pH of 3 whatever the incubation temperature. Growth optimum was determined at pH 5.5 and 24.5°C. Conditions where growth was not observed after 1 month incubation were considered not to allow fungal development as observed for pH lower than 3. Growth/no growth boundaries were compared with mycotoxin production/ no mycotoxin production boundaries for similar conditions on tomato purée based medium.

Significance: These results indicate the combination of pH and temperature where *Alternaria* mould development and mycotoxin production occurred. Knowing these boundaries will help industrials to optimize tomato product formulation and storage conditions to limit mould and mycotoxin development during shelf life.

P3-101 Genetic and Phenotypic Biodiversity of *Bacillus licheniformis* from the Dairy Industry

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Introduction: Among the Gram-positive, aerobic, spore-forming, food-spoiling bacteria, *Bacillus licheniformis* has a high prevalence in raw materials, ingredient and food, particularly in dairy products. Moreover, its properties of resistance to treatments, adhesion to surfaces and degradation of various substrates underlines potential abilities as a "super spoiler."

Purpose: The objective of this work is to evaluate genotypic and phenotypic biodiversity of this species by working on strains mainly isolated in the dairy industry but also foodborne strains from outside the dairy ecosystem as well as collections or epidemic strains. A better understanding of this diversity will allow better assessment of the spoilage risks linked to this microorganism.

Methods: More than sixty strains were studied by molecular methods: mainly PFGE and PCR based clustering analysis (REP PCR, M13 RAPD). Among these strains, about thirty were studied for their ability to produce biofilm, surfactant and different enzymes (gelatinase, caseinase, amylase and lipoprotease). All manipulations were performed at least in duplicate and the average result is used for further processing. Clusters of strains were made primarily by hierarchical clustering (Bionumerics, Minitab and JMP)

Results: Composite molecular fingerprint analysis generated by Bionumerix underlined a major group composed of the type strain, raw materials, environment and most dairy product isolates. In order to complete the picture of the potential risks, the combination of molecular and phenotypic diversity yields to a more diverse clustering. Even though in minority, a few strains were distinguished by their high potential for spoilage and/or biofilm formation.

Significance: These results underline the large diversity of strains of *B. licheniformis* encountered in dairy ecosystem as compared to strains from other origins. The study highlights the existence of clusters with similar behaviors or characteristics but also of more atypical strains that could be considered as "super spoilers."

P3-102 Genetic Diversity of *Clostridium* spp. Isolated from Spoiled Hard-cooked and Semi-hard Types of Cheese

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Introduction: Butyric acid fermentation, the late-blowing defect in cheese, caused by the outgrowth of Clostridia spores present in raw milk, can lead to considerable loss of product, especially in the production of semi-hard and Gruyère cheeses. Although *Clostridium tyrobutyricum* is the most frequently isolated strain from late-blown cheeses, spores of other clostridia, particularly *C. sporogenes, C. beijerinckii*, and *C. butyricum*, have also been isolated from natural and processed cheeses and raw milk. Conventional methods for the isolation of *Clostridium spp*. from cheeses with late-blowing defects are tedious and the identification of isolates is often complicated.

Purpose: The aim of this work was to develop and evaluate the use of molecular typing tools to detect and differentiate major species involved in late-blown cheeses.

Methods: A collection of over 300 Clostridia isolates was analysed using various molecular typing methods to perform clusters (REP-PCR) and assess genetic relatedness with a multi-locus sequence typing (MSLT) method targeting 5 different genes (*recA*, *groEL*, *tpi*, *rpoB*, *16S rDNA*). Specific PCR methods were developed i) to detect the major species involved in late-blown cheeses and ii) differentiate the major targeted species in particular distinguish closely related *C. sporogenes* and *C. botulinum*.

Results: 274 isolates were analysed using the REP-PCR method combined to the 16S rDNA sequencing (>600bp), providing more than 60 clearly differentiated groups. Several primer sets were designed to specifically detect *C. sporogenes, C. butyricum* and differentiate *C. botulinum* from other dairy-related Clostridia. Primers pairs were designed in the *recA* gene and the *tpi* gene for specific PCR detection of *C. sporogenes* and *C. butyricum,* respectively. A different primer pair was designed in the *recA* gene to specifically detect group A *C. botulinum*. The optimised protocols can distinguish the three target Clostridia species and no specific amplifications were obtained among other *Clostridium spp.* or non-target species (*Streptococcus thermophilus* and thermophilic *Lactobacilli*). The MLST of 16 *C. sporogenes* isolated revealed important intra-species diversity and locus frequencies that ranged from 4 to 8 alleles per locus. 11 unique profile patterns or STs were identified. The 16-23S rDNA PCR method yielded discriminative inter-species genomic fingerprint.

Significance: Clostridia strains isolated from raw milks and spoiled dairy products have been analyzed, leading to the set up of a characterized collection. Moreover, several methods have been developed to type and identify the isolates, respectively a REP-PCR fingerprinting method, as well as a MLST and species specific PCR methods.

P3-103 Ocurrence of Ochratoxin A-producing *Aspergillus* Section *Nigri* Strains from Brazilian Grapes

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Introduction: The species of *Aspergillus* section *Nigri* are major responsible for the contamination of grapes and wines with ochratoxin A (OTA). This mycotoxin is considered one of the most harmful to human health. It has been previously identified that fungal colonization and OTA production on grapes are influenced by several factors, including environmental conditions, location of the vineyard, soil type, and the characteristics of the grapes.

Purpose: The purpose of this study was to evaluate the incidence of black *Aspergillus* spp. in grapes from the tropical winemaking region of Brazil and their ability to produce OTA in Czapek yeast extract agar (CYA).

Methods: For this study, five grape cultivars were selected. A total of fifteen grape samples were harvested at maturity stage. The grape berries were directly plated onto DRBC and incubated for 7 days at 25 °C. After this time, all black *Aspergillus* spp. were isolated and identified by morphological characteristics. Their ability to produce OTA was quantified by HPLC with fluorescence detection.

Results: A total of 272 isolates of *Aspergillus* section *Nigri* were isolated and identified in the following species *A. carbonarius*, *A. niger*, *A. niger* Agregado, *A. foetidus*, *A. aculeatus* and *A. japonicus*. Among them, *A. niger* Agregado was the most common specie in all grape samplings (42%),

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followed by the *A. carbonarius* (38%). All isolates of *A. carbonarius* (103) were OTA producers in average levels (5-20 µg OTA/g CYA) and high levels (> 20 µg OTA/g CYA). The other species were not ochratoxigenic.

Significance: These data suggest that the potential risk of OTA contamination in grapes was associated with *A. carbonarius*, due to the high frequency of occurrence and the OTA levels produced in vitro.

P3-104 Identifying Molds Recovered from the Unopened Greek Containers by ITS1 Sequence Characterization

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Introduction: Recently, over 200 individuals have reported gastrointestinal illness after consuming the Greek yogurt in several states of United States. The consumers also reported bloating and swelling of some of the product containers. At first several unopened vials containing yogurt were examined for bacterial and fungal contaminations following conventional microbiological protocols. The recovered molds were genetically typed by ITS1 sequencing. The ITS1 locus has been considered as the "Universal DNA Barcode" to understand the fungal taxonomy.

Purpose: The major objective of this study was rapid species-identification of molds recovered from unopened vials with contaminated yogurt by ITS1 sequence characterization.

Methods: In this study, we have used our recently described protocols for DNA extraction, ITS1-specific PCR amplification and bi-directional nucleotide sequencing of PCR amplified ITS1 products for fungi species identification. The nucleotide sequencing data was analyzed using BioEdit and GENEIOUS programs.

Results: A total of 14 unopened yogurt vials were analyzed. Molds were recovered from all of these containers and PCR amplified at the ITS1 locus. Using ITS1 amplified products, the bi-directional DNA sequencing resulted high quality bases (> 98% HQ-100% HQ). Analysis of the generated ITS1 sequences confirmed species-identification to all recovered mold samples analyzed. The ITS1 nucleotide sequences obtained in this study matched 100% with the published sequence of *Rhizomucor variabilis* (GenBank Accession No. JF904893). The intra-specific genetic variation was not noticed.

Significance: The results suggest that the ITS1 locus is a reliable benchmark for rapid detection and differentiation of human-pathogenic fungi and molds. It will help in achieving the mission of our agency notably analyzing contaminated food with molds of public health importance.

P3-105 Meta-analysis of Heat Resistant Moulds Inactivation in Foods: Assessing Variability in Their Inactivation Kinetics and Impact on Food Spoilage

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Introduction: Heat resistant moulds (HRM) such as *Byssochlamys nivea, Byssochlamys fulva, Neosartorya fischeri* and *Talaromyces flavus* play a major role as spoilers of thermally processed fruit-based products.

Purpose: The purpose of this study was to conduct a meta-analysis on kinetic inactivation parameters of HRM in a variety of foods.

Methods: Data were obtained from scientific papers, dissertations and thesis. From a total of 45 scientific papers and 7 thesis/dissertations, 13 were selected because they either presented raw data on HRM inactivation kinetics, D-values or time-to-reduce a determined population. D-values (time required at specific temperature for 1 log reduction in the population of the microorganism) or δ (time for the first decimal reduction) at 85°C for different HRM in different substrates were compared.

Results: A total of 124 inactivation kinetics/D- or δ values were obtained for different HRM species and strains in several foods. Most data corresponded to inactivation kinetics/D- or δ values estimated using fruit juices or fruit-based fillings. Most inactivation kinetic data followed non-log linear inactivation kinetics. Overall, *N. fischeri* presented significantly higher D- or δ values at 85°C than *T. flavus* ($P < 0.05$) but not than *Byssochlamys* ($P > 0.05$). Average D- or δ values at 85°C found for *N. fischeri, Byssochlamys* and *T. flavus* were 1.60, 1.32 and 1.35, respectively. z-values ranged from 6.0°C, 6.5°C and 8.0°C for *Byssochlamys*, *N. fischeri* and *T. flavus*, respectively. Several factors were shown to affect HRM heat resistance.

Significance: The knowledge of variability in HRM heat resistance is of major importance for the design of thermal processes that ensure the inactivation of these microorganisms in foods. This is a growing concern because consumer's demand for mildly processed foods may increase the role and importance of HRM as food spoilers.

P3-106 A Mathematical Model to Predict Growth Probabilities of *Enterococcus* spp. in Low Sodium Chloride and Sodium Nitrite Processed Meat Products

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Introduction: Consumers prefer to have low sodium chloride and sodium nitrite processed meats, but *Enterococcus* spp. may proliferate in the products because of low concentration of low sodium chloride and sodium nitrite.

Purpose: This study developed mathematical models to determine optimum combinations of sodium chloride and sodium nitrite to prevent *Enterococcus* spp. growth in processed meat products.

Methods: A five-strain mixture of *Enterococcus* spp. was incubated in deMan, Rogosa and Sharpe (MRS) broth plus NaCl (0-1.75%) and sodium nitrite (0-120 ppm) at 4-15°C under aerobic and anaerobic conditions. Growth (referred to as 1) or no growth (referred to as 0) of *Enterococcus* spp. was determined by turbidity, and the growth response data were analyzed by the logistic regression to calculate the growth probability of the bacteria. The growth probabilities were validated to evaluate the model performance.

Results: In aerobic condition, at 4°C, the growth of *Enterococcus* spp. was not observed, but the growth of the bacteria in MRS broth was observed at 7-15°C, and higher sodium chloride concentration required low sodium nitrite concentration to inhibit *Enterococcus* spp. growth, and vice versa. However, at 120 ppm of sodium nitrite, sodium nitrite did not increase the inhibition of *Enterococcus* spp. For anaerobic condition, there was also no observation for *Enterococcus* spp. growth at 4°C, and at 7-12°C, *Enterococcus* spp. growth was inhibited at low sodium chloride concentration if sodium nitrite concentration was higher, and vice versa. At 15°C, single effect of sodium chloride on inhibition of *Enterococcus* spp. was not observed, but combination of sodium chloride and sodium nitrite inhibited *Enterococcus* spp. growth. Validation result suggested that the performance of developed probabilistic model was appropriate.

Significance: The probabilistic models developed in this study can determine optimum combinations of low sodium chloride and sodium nitrite concentration to inhibit *Enterococcus* spp. in processed meat products.

P3-107 Comparative Transcriptome Analysis of Sodium Nitrite Reduction in *Pseudomonas* spp.

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Introduction: Sodium nitrite plays a role as a color fixing agent and antimicrobial agent in processed meat products, but *Pseudomonas* spp. may reduce sodium nitrite in the meat products, which results in decreased activity of antimicrobial agent.

Purpose: The objective of this study was to elucidate if *Pseudomonas* spp. reduce sodium nitrite reduction and to analyze the gene related to sodium nitrite reduction.

Methods: A mixture of *Pseudomonas aeruginosa* (NCCP10250, NCCP10338, and NCCP11229) and a mixture of *Pseudomonas fluorescens* (KACC10326 and KACC10323) were incubated in nutrient broth (NB) plus 120 ppm sodium nitrite at 15°C for 216h. Residual sodium nitrite (ppm) in NB was calculated by diazotization reaction every 24h. After *P.aeruginosa* was identified for sodium nitrite reduction, transcriptome was analyzed by quantitative real-time PCR (qRT-PCR) to identify the sodium nitrite reductase gene for each *P. aeruginosa* strain.

Results: *P. aeruginosa* significantly decreased ($P < 0.05$) sodium nitrite concentration from 70 ppm to 0 ppm in NB after 168h of incubation, but *P. fluorescens* did not decrease sodium nitrite concentration compared to control (only sodium nitrite in NB). qRT-PCR analysis for three strains of *P. aeruginosa* strain showed that *nirS, norC, nosF,* and *nosZ* genes (sodium nitrite reductase gene) were expressed higher in NB plus 120 ppm sodium nitrite than that of control.

Significance: This result indicates that *P. aeruginosa* growth in processed meat products may allow foodborne pathogen growth by reducing sodium nitrite in the products.

P3-108 Modeling the Effect of Low Concentration of NaCl and $NaNO_2$ on the Growth Probability of *Pseudomonas* spp

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Introduction: Recently, the consumers' demand for low NaCl and $NaNO_2$ processed meat products has been increased, but microbial safety of the meat products has not been fully studied.

Purpose: This study developed probabilistic models to determine the optimum combinations of NaCl and $NaNO_2$ to produce growth-no growth interface of *Pseudomonas* spp. growth.

Methods: The combinations of NaCl (8 concentrations; 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, and 1.75%) and $NaNO_2$ (9 concentrations; 0, 15, 30, 45, 60, 75, 90, 105, and 120 ppm) were prepared in nutrient broth, and placed in 96-well microtiter plate, followed by inoculation of a five-strain mixture of *Pseudomonas* spp. in each well. All microtiter plates were aerobically incubated at 4-15°C for 144-528h. Growth (referred to as *1*) or no growth (referred to as *0*) for each combination was then determined every 24h by turbidity. The growth response data were then analyzed by the logistic regression to produce probabilistic model for *Pseudomonas* spp. growth. Eventually, the predicted growth probabilities were compared to the observed growth to evaluate model performance.

Results: NaCl and $NaNO_2$ were significantly effective ($P < 0.05$) on inhibiting *Pseudomonas* spp. growth during storage at 4-12°C. The combination matrix composed of NaCl and $NaNO_2$ concentrations was prepared by the developed probabilistic model, and the result showed that at low NaCl concentration, higher $NaNO_2$ level was required to inhibit *Pseudomonas* growth at 4-15°C. However, at 15°C, there was no significant effect of NaCl and $NaNO_2$. A validation result showed that the performance of the probabilistic model developed in this study was appropriate.

Significance: The developed probabilistic models in this study should be useful in determining optimum conditions for low NaCl and $NaNO_2$ to inhibit *Pseudomonas* spp. growth in processed meat products.

P3-109 Reduced Time to Detection of *Alicyclobacillus acidoterrestris* in Fruit Juice Concentrates Using the Pall GeneDisc® Rapid Microbiology System

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Introduction: *Alicyclobacillus acidoterrestris* is an acidophilic, thermophilic, gram-positive bacterium that causes spoilage of fruit juices due to endospore survival of pasteurization. Ingredient risk assessment is part of an overall strategy to prevent *A. acidoterrestris* spoilage. However, traditional culture-based methods may take over a week, causing production delays, product holds or supply chain disruptions.

Purpose: This study compared the time needed to detect *A. acidoterrestris* spores in juice concentrates using culture-based methods and the GeneDisc System—a real time, quantitative, PCR-based system.

Methods: Apple and orange juice concentrates were inoculated in triplicate with *A. acidoterrestris* spores at 0.5 spores/ml and 0.1 spores/ml. Concentrates were diluted 1:10 with water (apple) or YSG broth (orange) and heat shocked at 80°C for 10 min. Orange juice was incubated at 43°C and sampled daily. Apple juice was filtered through a 0.45 µm pore-size filter. The filter was placed in 10 ml YSG broth, incubated at 43°C and sampled daily. For analysis by culturing, samples were plated on YSG agar and incubated at 43°C. For analysis, DNA was extracted from samples and amplified per the system protocol.

Results: Samples spiked with 0.5 spores/ml were detected positive by the system after a 24-hour enrichment. Cycle threshold (Ct) values were 13.43 ± 0.96 for apple juice and 27.67 ± 3.45 for orange juice (mean ± sd). Using culturing, *A. acidoterrestris* was not detectable until at least 48 hrs. Samples spiked with 0.1 spores/ml were detected positive by the system after a 48-hour enrichment. Ct values were 15.50 ± 1.23 for apple juice and 31.27 ± 6.15 for orange juice. Detection by culturing required 48 hrs for apple juice and up to 5 days for orange juice.

Significance: The system can substantially reduce the time needed to detect *A. acidoterrestris* contamination of fruit juice concentrates.

P3-110 Effect of Acetic and Citric Acids on the Growth of *Bacillus licheniformis* in Cucumber Juice Medium

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Introduction: *Bacillus licheniformis* has been shown to cause pH elevation and metabiotic effects in tomato products, which contain citric acid and have pH values around 4.6. The organism may pose a potential risk to acidified vegetable products which typically contain acetic rather than citric acid.

Purpose: To compare the effects of citric and acetic acid on the growth of *B. licheniformis* for pH values between 4.6 and 7, and determine if *B. licheniformis* growth may occur in vegetable products containing acetic rather than citric acid.

Methods: Cocktails of 3 to 5 strains of *B. licheniformis* were grown Tryptic Soy Broth or cucumber juice (CJ), a non-inhibitory vegetable growth medium in microtiter plate wells (200 µl volumes). Plates were incubated with lids at 30°C for 24 hr to determine growth rates by optical density. For acid concentrations ranging from 25 mM to 300 mM, growth rates were compared to protonated and total acid concentrations.

Results: For pH 4.6, the highest total concentration of citric acid tested (300 mM) was approximately 8 times less inhibitory compared to the lowest total acetic acid concentration (25 mm). For both acids, combined data for all pH and acid conditions indicated that 10 to 15 mm (fully) protonated acid was needed to inhibit growth.

Significance: The data indicate that acetic acid was significantly more effective at inhibiting *B. licheniformis* growth than citric acid for conditions typical of acidified vegetable products. These data could help explain why pH increase due to *B. licheniformis* has not been seen in pickled vegetables but has been observed in tomato products.

P3-111 Toward a Global Approach in Food Preservation by Investigating the Physiological Behavior of *Geobacillus stearothermophilus*

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Introduction: *Geobacillus stearothermophilus* is recognised as the most prevalent flat-sour organism in the canning industry due to the high heat resistance of its spores. To control its spore's high heat resistance, the heat treatment intensity could be associated with environmental conditions.

Purpose: The purpose of this work was to study the effect of temperature and pH on the germination and the growth of spores produced at different sporulation conditions. A global model was proposed based on a single set of parameters, commonly named growth cardinal values.

Methods: The influence of temperature and pH on the bacterial growth, recovery and sporulation abilities of *Geobacillus stearothermophilus* ATCC 12980 was studied on nutrient agar. Growth cardinal values and heat resistance ($D_{115°C}$) were estimated at different sporulation and recovery conditions.

Results: Based on experimental results, growth cardinal values were estimated by fitting the cardinal model at 38.52°C, 57.59°C, 68.02°C, 5.27, 7.17 and 8.91 respectively for T_{min}, T_{opt}, T_{max}, pH_{min}, pH_{opt} and pH_{max}. D-values obtained at different temperatures and pH of sporulation and recovery showed that the highest heat resistances are obtained in conditions allowing optimal growth and these D-values decreased at suboptimal growth pHs and temperatures. The current observations revealed also that sporulation and recovery occurred only in the range of temperature and pH allowing the growth, therefore a model was proposed to describe heat recovery using growth cardinal values.

Significance: This work provides a new global approach in food preservation taking into account the sporulation history and the recovery conditions. Growth cardinal values could be the unique set required to model and to predict heat resistance.

P3-112 *Streptococcus infantarius* Strains Isolated from Brazilian Goat Milk

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Introduction: *Streptococcus infantarius* is a species isolated from dairy products and infants and is considered as opportunistic pathogen.

Purpose: Are *St. infantarius* isolates able to survive in the human GIT, to compete with LAB and to present risk for consumption of contaminated milk?

Methods: Isolates K1-4 and K5-1, differentiated by RAPD-PCR have been investigated for their ability of aggregation to intestinal pathogens; de-conjugation of TCA, TDC, GCA and GDC; hydrophobicity; survival under conditions simulating the GIT; and resistance to antibiotics.

Results: Both *St. infantarius* showed good survival rates when exposed to simulating gastric and enteric conditions (86.9% and 69.9% for K1-4 and 25.9% and 68.6% for K1-5). Both *St. infantarius* were able to grow in presence of TDCA, TCA, GDCA or GCA, but did not show ability to de-conjugated bile-salts. *St. infantarius* K1-4 and K5-1 exhibited 25.4% and 89.9% hydrophobicity, and 67.9% and 23.0% auto-aggregation. *St. infantarius* K5-1 showed production of β-galactosidase, however, *St. infantarius* K1-4 was negative. The strain K1-4 showed resistance (MIC) to imepinem (0.5 µg/ml), vancomycin (2.0 µg/ml), ampicillin (0.25 µg/ml), clindamycin (4.0 µg/ml), tetracycline (0.25 µg/ml), erythromycin (0.25 µg/ml) and gentamicin (32 µg/ml). *St. infantarius* K5-1 presented similar resistance to antibiotics: imepinem (0.15 µg/ml), vancomycin (1.0 µg/ml), ampicillin (0.6 µg/ml), clindamycin (0.15 µg/ml), tetracycline (12 µg/ml), erythromycin (0.06 µg/ml) and gentamicin (32 µg/ml). High levels of co-aggregation with *E. coli*, *L. monocytogenes*, *C. perfringens* and *Salmonella* spp. have been recorded for both isolates.

Significance: According to the results, *St. infantarius* K5-1 present better potential to colonize and compete with GIT microflora compared to *St. infantarius* K1-4. Both strains presented resistance to the major groups of antibiotics. Milk contaminated with this two strains presenting a health risk since the studied *St. infantarius* strains presented good ability to survive to simulated GIT conditions and to be responsible for a chronicle GIT disorders.

P3-113 Development of Probabilistic Model to Predict *Lactobacillus* spp. Growth as a Function of NaCl and $NaNO_2$

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Developing Scientist Competition

Introduction: NaCl and $NaNO_2$ have been commonly used in processed meats, and *Lactobacillus* spp. are commonly isolated from the products. However, the effect of NaCl and $NaNO_2$ on *Lactobacillus* spp. has not been evaluated

Purpose: This study developed probabilistic models to evaluate the effect of NaCl and $NaNO_2$ on *Lactobacillus* spp. growth.

Methods: A five-strain mixture of *Lactobacillus* spp. was inoculated in MRS (deMan Rogosa and Sharpe) broth supplemented with NaCl (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, and 1.75%) and $NaNO_2$ (0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, and 210 ppm), and all samples were aerobically and anaerobically stored at 4-15°C up to for 816h. Growth (assigned the value of 1) or no growth (assigned the value of 0) for each combination was determined by turbidity every 24h. These growth response data were then analyzed by the logistic regression to calculate the growth probability of *Lactobacillus* spp. as a function of NaCl and $NaNO_2$at each temperature. Subsequently, validation was conducted to evaluate the model performance.

Results: For aerobic storage, NaCl and $NaNO_2$ significantly ($P < 0.05$) inhibited *Lactobacillus* spp. growth at 4, 7, 10, and 12°C, and the obvious synergistic effect of NaCl and $NaNO_2$ was also observed to inhibit *Lactobacillus* spp. However, the synergistic effect was not observed at 15°C. In anaerobically stored samples, the significant effect of NaCl and $NaNO_2$ was observed at 4, 7, and 10°C, and the synergistic effect was of NaCl and

$NaNO_2$ was observed up to at 12°C. Validation result showed that the developed models were appropriate to predict the growth probability of *Lactobacillus* spp. at different concentrations of NaCl and $NaNO_2$.

Significance: The probabilistic models developed in this study should be useful in determining optimum conditions to inhibit *Lactobacillus* spp.

P3-114 Internalization of Shiga Toxin-producing *Escherichia coli* in Beef Products as Influenced by Vacuum Marination

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Introduction: Though successful in improving palatability, the risk of pathogen internalization propagated by vacuum-tumbled marination has been documented. As concerns regarding non-O157 Shiga toxin-producing *E. coli* serogroups rise—particularly those referred to as the "Big Six," data regarding the internalization in marinated meat products is important.

Purpose: The purpose of this study was to investigate the internalization and translocation of non-O157 and O157:H7 Shiga toxin-producing *Escherichia coli* in vacuum marinated beef products.

Methods: This study evaluated the marinade-mediated internalization and translocation of seven individual STEC serogroups (O157:H7, O26, O45, O103, O111, O121, and O145) in surface inoculated beef sirloin flaps. Beef bottom sirloin flaps were inoculated (10^6 log CFU/cm^2) with a multi-strain cocktail prior to vacuum tumbled marination (30 or 60 min) with a standard marination solution containing a food-grade dye and refrigerated storage (14 d). Pathogen presence and marinade migration after marination and storage were evaluated.

Results: Data suggest that dye penetration was enhanced with vacuum storage for 14 d. Marination length had no influence on the immediate (d 0) internalization of STEC in sirloin beef flap sections, except for O145 ($P = 0.03$), which was greater after 60 min of tumbling. Marination for as little as 30 min resulted in approximately 10^4 log CFU/g of internalized STEC, but, marination length did not influence ($P > 0.05$) the population of STEC present on the subprimal surface immediately after vacuum marination (d 0) or after 14 d of storage.

Significance: Marination length did not influence STEC attachment and had little influence on pathogen internalization; however, 30 min of vacuum marination resulted in substantial pathogen internalization. Likewise, increased marination length (60 vs. 30 min) increased ($P < 0.05$) the percentage of marinade uptake, regardless of inoculation serogroup.

P3-115 Detection of *Salmonella* from Cloves

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Introduction: FDA recall and outbreak data clearly indicate that spices and seasonings are vehicles for salmonellosis. Detection of *Salmonella* in some spices, such as cloves and cinnamons, remain a challenge, due to natural antimicrobial substances in these spices.

Purpose: To develop an effective detection method for *Salmonella* from spices with antimicrobial substances, using clove flower buds as a model.

Methods: Two clove varieties, Ceylon and Madagascar, were used in the study. Cloves were inoculated with *Salmonella* Montevideo, *Salmonella* Typhimurium, or *Salmonella* Weltevreden at 1, 3 or 6 log CFU/g. Two sample sizes, 10 and 25g, were compared. Trypticase soy broth (TSB) was used as preenrichment broth at a broth:clove ratio of 9:1. After adding TSB to the weighed cloves for preenrichment, three different preenrichment treatments were evaluated: cloves were left in the preenrichment TSB broth during preenrichment (preE 1); the mixture was shaken vigorously for 30 s and decants were transferred to a new bag for preenrichment (preE 2); and the mixture was shaken vigorously for 60 s and decants were transferred to a new bag for preenrichment (preE 3). The rest of the procedures were carried out according to the FDA BAM. Five trials were conducted so far and each trial had 4 replicates.

Results: Combining the data from the 5 trials together, all 10 and 25g samples from preE 2 and preE 3, at inoculation levels 6 log CFU/g or 3 log CFU/g, were positive for *Salmonella*. However, treatment preE 1 only produced 30 positive results among a total of 40 samples, at inoculation levels 6 log CFU/g; it had 12 positive results among 40 samples, at inoculation levels 3 log CFU/g. At inoculation levels 1 log CFU/g, only 1 of 40 samples tested was positive for *Salmonella* by preE 1; 17 of 40 positive by preE 2; and 18 of 40 positive by preE 3. The results clearly indicated that a modified soak procedure, where cloves were removed from the preenrichment broth after 60 sec, can greatly improve the sensitivity of the detection method.

Significance: This discovery reduced the detection limit for *Salmonella* on clove flower buds from 3 log CFU/g to 1 log CFU/g.

P3-116 Evaluation of a Novel Time-temperature Indicator Using Maillard Reaction for Food Safety

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Introduction: Many kinds of time-temperature indicators (TTIs) have been developed for confirming the temperature histories of food products during the storage and transportation. Recently Yamamoto and Isshiki (2012) developed a novel TTI using Maillard reaction. The reaction starts by the mixture of xylose and glycine solutions. The color of the indicator changed from colorless to blue, and finally dark brown, which is very easy to recognize.

Purpose: This study was aimed to kinetically evaluate the characteristics of the indicator at various patterns of temperature with a mathematical model. Microbial growth data previously published was also used for evaluation.

Methods: The indicator was stored at constant and dynamic temperatures in an incubator and the color of the indicator (delta E) was measured with a Tristimulus colorimeter (Konica-Minolta, Japan) with intervals during the storage. The color change was analyzed with a mathematical model. The growth of *Pseudomonas fluorescens*, a psychrophile in sterile chicken at various patterns of temperature was measured with a plating method. Microbial growth data at various patterns of temperature reported in published papers were also analyzed in terms of time and temperature.

Results: The color change of the indicator at a constant temperature ranging from 4°C to 32°C during the storage was successfully described with the model. With those data, the color change of the indicator at dynamic temperatures was precisely predicted. At the times at various patterns of temperature to reach a given color change of the indicator, the increase in microbial population in food was almost constant.

Significance: This study showed that the color change of the indicator successfully estimated microbial growth in food at various patterns of temperature, suggesting that it could be a useful TTI.

P3-117 Prediction of Microbial Shelf Life of Raw Chicken Breast under Aerobic Storage Conditions Using Volatile Spoilage Biomarkers

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Introduction: Fresh, raw chicken breast is a highly perishable food, of great importance, characterized by complex microbial ecosystem. Microorganisms responsible for spoilage are known as specific spoilage organisms (SSOs), and their activity is commonly manifested as off-odors caused by the presence of volatile organic compounds (VOCs) produced as a result of bacterial metabolism.

Purpose: The aims of this investigation were to find unique VOCs as possible spoilage biomarkers to predict microbiological spoilage of raw chicken breast stored aerobically under different temperature conditions and to characterize SSOs growth dynamics.

Methods: The growth of total viable count (TVC) and six SSOs including: psychotropic bacteria, *Pseudomonas* spp., coliforms, lactic acid bacteria (LAB), *Brochothrix thermosphacta* and H_2S producing bacteria were investigated. The VOCs profiles were determined by HS-SPME-GC-MS. Chicken breast samples were stored at 4 and 10°C for 14 days and 21°C for 3 days. The growth curves were fitted to the modified Gompertz model. The predicted shelf life was defined by the time, when TVC reached 7.0 log CFU/g. Maximum concentration level coefficients (M values) for the growth of SSOs were designated. The results were statistically analyzed by ANOVA followed by Tukey's significant difference test.

Results: Regardless of the temperature, predominant SSOs (highest M values) in naturally spoiled chicken breast were: psychrotrophs, probably represented mostly by *Pseudomonas* spp., followed by LAB. The predicted shelf lives of chicken breasts were 1.95, 1.20 and 0.29 days for storage at 4, 10 and 21°C, respectively. The significant ($P < 0.05$) and high correlations between TVC and SSOs, and selected VOCs (ethanol, 1-butanol-3-methyl and acetic acid) were observed in all studied conditions.

Significance: This study suggests that the identified VOCs could be used as the most promising volatile spoilage biomarkers for chicken breast.

P3-118 Microbial Quality of Fish and Game Meats Preserved by First Nation's Traditional Smoking Method

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Introduction: First Nation's communities rely on traditional preservation methods (i.e., smoking and drying) for game and fish to ensure the long term availability and safety of their food supply. Currently, few data exist regarding the microbial safety and quality of these smoked foods, suggesting the efficacy of traditional practices to produce safe food should be assessed.

Purpose: The aim of this study was to evaluate the microbial quality and safety of fish and game preservation methods practiced by a northern BC First Nation's community.

Methods: In total, 44 fish and game samples were obtained. Quality indicators including total aerobic bacteria (TAB), coliforms, generic *Escherichia coli*, lactic acid bacteria (LAB), *Staphylococcus aureus*, and yeast/molds were enumerated using the Tempo® system. Additionally, the Vidas® system was used to detect the pathogens *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. The opportunistic pathogens *Enterococcus faecalis* and *E. faecium* were detected using culture methods with subsequent PCR confirmation. Comparisons between samples were performed using *t*-tests.

Results: Raw moose samples contained significantly ($P < 0.05$) higher levels of all quality indicators compared to raw fish. Significantly ($P < 0.05$) higher levels of TAB, coliforms, LAB, and yeasts/molds were observed in smoked fish when compared to raw fish (fully dried products excluded). No differences ($P > 0.05$) were found between raw and smoked moose samples. Fifty-seven percent of smoked products and 50% of raw moose samples contained >5 log CFU/g of TAB and 71% and 50% of raw moose samples contained >4 log CFU/g of coliforms and *E. coli*, respectively. *E. faecalis* was isolated from five raw and five smoked samples and *E. faecium* from one raw sample. No other pathogens were detected.

Significance: The results from this study will assist First Nation's communities in improving sanitary and hygienic conditions of existing production practices for smoked food products resulting in increased food security and long-term well-being.

P3-119 Perishable Meat and Seafood Products Ordered from Online Vendors in the United States – Analysis of Shipping Methods, Packaging Materials, and Product Temperatures

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Introduction: Perishable food products marketed directly to consumers by online vendors are delivered using the same methods as non-perishable products, making them vulnerable to temperature abuse.

Purpose: This study reports on shipping methods, packaging, coolant materials, and product temperatures of perishable meat, fish, and seafood products ordered online.

Methods: Samples of 679 raw meat, poultry, fish, and shellfish products were purchased online from 160 US purveyors between January and October 2013, and shipped using common carriers.

Results: Including nine replacements, 169 shipments were received and 684 food items were tested. Of these, 375 were meat, 47 poultry (chicken, duck, turkey), 231 seafood (finfish and shellfish) and 30 "other" products. Orders were shipped via FedEx (59.2%), UPS (39.6%); or USPS (<1%). Shipping was 'overnight' (32.4%), '2-day' (29.4%), and 'standard/ground' (27.1%). Mean transit time was 32.4 hours (*SD*=14.8 hours). Only 4.1% had external damage, although 3.6% had leakage on the inside. Only 36.7% had food safety information on the outside of the box; 7.7% had no labels indicating their perishable contents. Only 25% had any food safety information inside the package. The majority of shipments (92.9%) included a polystyrene foam box; 76.9% also used an exterior cardboard box. The majority (50.6%) contained gel packs, 42.4% had dry ice, 4.1% included both; 2.4% contained conventional ice, and 4.1% of the packages contained no refrigerant. Upon opening the packages, the surface temperatures on the top of the products ranged from –23.5°F to 72°F (M = 36.8°F, *SD* = 13.3°F). The temperatures on the bottom ranged from –22.7°F to 75°F (M = 36.8°F, *SD* = 12.9°F). Nearly half (46.6%) had a surface temperature above 40°F.

Significance: Many of the perishable meat, poultry, and seafood products arrived outside of the temperature safety zone. This, combined with a lack of food safety information accompanying the packages, places consumers at increased risk for foodborne illness.

P3-120 Microbiological Evaluation during the Processing of Heart of Palm

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Introduction: Brazil is one of the major suppliers of heart of palm in the world. The heart of palm is the large meristem of diverse palm species. It is cut in sticks or pickled and canned for local and international commerce. The hearts of palm are sterile at cutting, but handling exposes them to microorganisms. To ensure the safety, pasteurization must be carried out.

Purpose: This research was done to evaluate which were the steps that promote the major microbiological contamination during the processing.

Methods: The heart of palm process of a big processing industry had the following steps: I) receipt of the heart of palm; II) removing of the sheaths; III) cutting or picking the shoots; IV) washing; V) arranging in bottle; VI) adding solution; VII) exhausting; VIII) sealing; IX) pasteurization; X) storage.

Using specific Petrifilm from 3M, the following microbiological analyses were done after steps III; V; and IX: aerobic mesophilic bacteria, fecal coliform, *Staphylococcus* spp., mold and yeast.

Results: Initial population (step III) was: 4.7 x 10^8 CFU/g for aerobic mesophilic bacteria; 1.2 x 10^5 CFU/g for fecal coliforms; 2.9 x 10^5 CFU/g for *Staphylococcus* spp.; 5.9 x 10^5 CFU/g for mold and yeast. After step V: 7.7 x 10^6 CFU/g for aerobic mesophilic bacteria; 2.6 x 10^5 CFU/g for fecal coliforms; 1.2 x 10^4 CFU/g for *Staphylococcus* spp.; 2.2 x 10^4 CFU/g for mold and yeast. The reductions observed were obtained due the washing step. After pasteurization the population observed was: 2.9 x 10^3 CFU/g for aerobic mesophilic bacteria; none fecal coliforms; 3.2 x 10^2 CFU/g for *Staphylococcus* spp.; 2.3 x 10^3 CFU/g for mold and yeast.

Significance: The results suggest that some improvement must be done during steps I and II to ensure a lower microorganism level in the other steps of the processing.

P3-121 Validation of Rehydratable Dry-film Aerobic Count (AOAC 990.12) and Total Coliform (AOAC 991.14) Methods for Determination of Microbiological Quality of Margarine

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Introduction: Margarine is a liquid emulsion of plastic consistency, usually water/oil obtained especially from non-dairy edible fats and oils with a minimum fat content of 80%. From a public health and quality assurance perspective, testing margarine for total bacterial counts and coliforms provides an indication of hygienic processing conditions and potential for post-processing contamination.

Purpose: Validate rehydratable dry-film microbiological test methods for determination of Aerobic Bacteria (AOAC 990.12) and Total Coliforms (AOAC 991.14) in margarine against conventional media to assess validity and enhance credibility and confidence in the results obtained with these alternative methods.

Methods: A margarine matrix was inoculated with *Escherichia coli* ATCC 25992 at levels of 34 or 44 CFU/g and ten samples per media type (n = 20) were plated in duplicate according to AOAC 990.12 and AOAC 991.14 methods, respectively. Plate count agar plates (n = 20) were inoculated in duplicates at the same levels. Sterility of the margarine matrix was assessed by plating ten uninoculated margarine samples on each media type. All samples were incubated at 35°C. The results were compared to internal validation criteria for accuracy, reproducibility and uncertainty.

Results: There was no significant difference in microbial recovery from the margarine matrix observed between traditional media and methods AOAC 990.12 (P = 0.702) or AOAC 991.14 (P = 0.565). Results fell within established internal parameters for accuracy, reproducibility and uncertainty.

Significance: Methods AOAC 990.12 and 991.14 were validated internally as accurate, precise and as carrying an acceptable level of uncertainty when analyzing a margarine sample matrix. Rehydratable dry-film media constitutes a reliable and convenient alternative with validity comparable to traditional media for determining the microbiological quality of margarine.

P3-122 Efficacy of GRAS Products against *Listeria monocytogenes* in Pure Culture and on Raw Salmon Fillets

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Introduction: *Listeria monocytogenes* (Lm) is associated with humans, domestic animals, agricultural products, fresh and saltwater environments, and food processing plants. Raw fish such as, salmon, can contain Lm and become contaminated with Lm from contact with food processing surfaces. Lm has shown resistance to preservatives, antibiotics, and sanitizers, and can grow at refrigeration temperatures.

Purpose: We examined the ability of GRAS (generally recognized as safe) products to inhibit growth of pure cultures of Lm and Lm on refrigerated raw salmon fillets.

Methods: Two GRAS products (2% and 4% DV™ [vinegar-based] and 1% ProTek™ [NaCl:acetate:diacetate]) were evaluated using Bioscreen C (TSB + 0.1% yeast extract, 30°C, 18h) for effectiveness against pure cultures of Lm (strains 424, 427, 2307, 3371, 3382; 5 log CFU/ml). Subsequently, 25-g portions of raw salmon (3-6 replicates) were surface-inoculated with Lm strain 3382 at 4 log CFU/g and treated with sterile deionized water (DI, control) and 15% vinegar-based solution (≤ 2% pick-up) using a prototype sprayer. Lm counts were determined by spread-plating pulsified fillets on R&F chromogenic agar (30°C, 24-72h) immediately following treatment and at weekly intervals during cold storage (4°C, 4 weeks).

Results: With Bioscreen C, the greatest reduction in Lm growth (turbidity), 82-88%, was produced by 4% vinegar-based; growth was reduced 34-41% by 2% vinegar-based and 20-29% by 1% NaCl: acetate:diacetate. On raw salmon, vinegar-based prevented Lm growth for one week at 4°C; Lm numbers increased by 0.5 log CFU with DI treatment. Lm increased by 0.7 and 1.6 log CFU on vinegar-based- and DI-treated salmon, respectively, after two weeks at 4°C. Lm numbers were 1 $\log_{10}$CFU less on vinegar-based-treated salmon vs. control samples after four weeks at 4°C (P< 0.05).

Significance: These findings suggest that vinegar-based could be used as a listeristatic (<1 log increase) control measure during processing to reduce contamination of refrigerated raw salmon.

P3-123 Occurrence of *Listeria* spp. and Characterization of *Listeria monocytogenes* from a Fish Processing Facility in British Columbia, Canada

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* is a foodborne pathogen, which can cause a severe disease, listeriosis, in at-risk populations. A recent survey of ready-to-eat (RTE) foods and production environments in British Columbia (BC) detected *L. monocytogenes* in fish facilities and in RTE fish products destined for market. Given strict regulations pertaining to *L. monocytogenes* in RTE foods, *L. monocytogenes* contamination presents a challenge for BC RTE fish processors.

Purpose: To determine the occurrence of *Listeria* spp., including *L. monocytogenes*, in a BC RTE fish processing plant and characterize recovered *L. monocytogenes* genetically and phenotypically.

Methods: Environmental samples (n = 1748) were collected over a one-year period from a RTE fish processing plant in BC and were subjected to Health Canada standard procedures MFHPB-29 or MFHPB-30 for detection and isolation of *Listeria* spp. and *L. monocytogenes*. *L. monocytogenes* isolates (n = 14) were subsequently characterized by lineage-typing ASO-PCR, assessed for frequency of mutation by plating isolates on Brain Heart Infusion Agar with rifampicin (100µg/ml), and screened by PCR for genetic markers that may contribute to increased persistence (e.g., LGI1 and *bcrABC)*.

Poster

Results: *Listeria* spp. and *L. monocytogenes* were recovered in 2.6% and 1.0% of samples, respectively. Cutting boards and raw fish surfaces yielded 93% of the samples positive for *L. monocytogenes*. Lineage-typing revealed that the majority of *L. monocytogenes* recovered belong to Lineage I (60%), with the remainder belonging to Lineage II. The frequency of mutations between lineages was not significantly different (P = 0.07) and LGI1 and *bcrABC* were absent in all screened isolates.

Significance: This research provides improved understanding of *Listeria* spp. and *L. monocytogenes* prevalence in a BC RTE fish processing facility coupled with details regarding genetic and phenotypic attributes of recovered *L. monocytogenes*. This knowledge will help processors tailor their *L. monocytogenes* control strategies to minimize contamination of products entering the food chain.

P3-124 Validation of the New Rapid Detection System - 3M Petrifilm *Salmonella* Express System for the Detection of *Salmonella* in Raw and Processed Seafood Products

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Introduction: Seafood products are a major food category for Thailand's export. *Salmonella* contamination in seafood products has been frequently reported, raising a global public health concern. A new rapid detection system, 3M™ Petrifilm™ *Salmonella* Express (SALX) System, employs a simpler all-in-one method (sample enrichment to confirmation of results) for screening of *Salmonella* in food products, and yields faster and reliable results.

Purpose: This study evaluated the performance of the system for the detection of *Salmonella* in a variety of raw and processed seafood products from Thailand as compared to the standard ISO 6579-1:2007 method.

Methods: Products (n = 200) were purchased, as manufacturer-sealed packaging or individually weighed/sliced from retail counters, markets, and street vendors. Products included 43 raw seafood products, 66 processed (ready-to-cook) seafood products (e.g., dried assorted fish), and 91 RTE products (e.g., mixed fish wonton). Each sample tested (approx. 100 g) was homogenized and screened for *Salmonella* following two methods; the system and ISO 6579-1:2007.

Results: The system showed comparable detection results to those obtained from the ISO method. Overall, 112/200 samples were positive for *Salmonella* (56%). Twelve samples tested positive by the system only. These samples encompassed a variety of different food matrices; six were products with high microbial loads. The sensitivity of the system was 100% (95% CI: 95.4-100%), specificity was 88.0% (95% CI: 81.6-94.4%), accuracy was 94.0%, and the positive predictive value was 89.3% (95% CI: 83.6-95.0%).

Significance: Rapid detection and monitoring of *Salmonella* in seafood products can improve seafood safety and quality in domestic and international markets. The 3M Petrifilm SALX System is a new all-in-one detection method that can facilitate high-throughput sample screening for *Salmonella* to obtain faster and reliable results.

Poster

P3-125 Mercury in U.S. Commercial Finfish and a Fish Size-Mercury Concentration Correlation for Swordfish

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Developing Scientist Competition

Introduction: Mercury, a byproduct of industrial waste and combustion processes, is biotransformed to methylmercury (MeHg) by aquatic microbes and bioaccumulates in fish. Fetuses and infants are especially sensitive to the neurotoxic effects of MeHg, as excessive exposure can impede the development of language, attention, memory, motor skills and learning ability. Because fetal and infant intake is dependent upon the maternal diet, pregnant and nursing women should exercise caution when consuming fish, as some species contain high MeHg concentrations.

Purpose: The purposes of this study were 1) to examine the mercury content in many commercial finfish species commonly consumed in the U.S. and 2) to develop a fish size-mercury concentration correlation for swordfish.

Methods: Commercial seafood vendors from six regions of the U.S. (Great Lakes, Mid-Atlantic, New England, Northwest, Southeast, and Southwest) provided 77 species of fish (300 composites of three fish). Total mercury content was determined using a thermal decomposition (gold) amalgamation atomic absorption spectrophotometer (TDA/AAS) mercury analyzer (DMA-80), which thermally decomposes samples, captures mercury via gold amalgamation and measures total mercury content via AAS.

Results: Fish muscle tissue contained mercury concentrations ranging from 1 – 1425 ppb. The most popularly consumed species, including salmon (13–62 ppb), Alaskan Pollock (11 ppb), tilapia (16 pp), channel catfish (1 ppb), Atlantic cod (82 ppb) and pangasius/swai (2 ppb), were low in mercury, while king mackerel (1425 ppb) and swordfish (1107 ppb) were above the current FDA Action Limit of 1000 ppb. Swordfish was further investigated and it was determined that dressed carcasses above 230 lbs. exceeded the FDA limit.

Significance: The results of this study will help sensitive populations make safe and informed decisions regarding seafood consumption. This survey will also help regulatory agencies as they prevent fish with excessive mercury from entering the marketplace.

P3-126 Inactivation of Murine Norovirus and Feline Calicivirus during Oyster Fermentation

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Introduction: Fermented oysters, called '*eoriguljeot*', are popular representative fermented seafood in Korea. While reduction of foodborne pathogens in fermented foods has been well studied, the survival of foodborne viruses in fermented foods is not well understood. As many norovirus (NoV) outbreaks were associated with the consumption of raw oysters, it is important to ensure the microbial safety of fermented oysters.

Purpose: The aims of this study were to examine the survival of norovirus surrogates including feline calicivirus (FCV) and murine norovirus (MNV) and to investigate the change of pH and enzyme production during oyster fermentation.

Methods: Oysters spiked with FCV or MNV were fermented with 5% or 10% salt at 18°C for 15 days. The titers of MNV and FCV, populations of lactic acid bacteria (LAB), pH, and enzymatic activity were measured at 0, 1, 3, 5, 7, 10, and 15 days post-fermentation (DPF).

Results: Oysters supplemented with 5% NaCl showed more rapid fermentation than those supplemented with 10% NaCl. In 5% NaCl-supplemented oysters at 15 DPF, MNV and FCV titers were significantly decreased by 1.60 log and 3.01 log, respectively. As the populations of LAB increased up to 8.77 log CFU/g during oyster fermentation, pH gradually decreased to 4.17. The production of α-amylase, protease, and lipase were significantly higher in 5% salted oysters than in 10% salted oysters.

Significance: MNV and FCV were significantly reduced during oyster fermentation. The combination of LAB, low pH, and digestive enzymes produced in fermented oysters could contribute to reduce norovirus surrogates.

P3-127 Effects of Ambient Air Storage and Resubmersion of Oysters on *Vibrio vulnificus* and *Vibrio parahaemolyticus* Levels

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Introduction: *Vibrio vulnificus* (Vv) and *Vibrio parahaemolyticus*(Vp) are the two leading causes of bacterial illnesses associated with raw shellfish consumption. Levels of these pathogens can increase in oysters held under warm, ambient air conditions, as in certain routine aquaculture practices. To reduce Vv and Vp levels oysters are subsequently resubmerged prior to final harvest.

Purpose: This study sought to identify changes in Vv, and total and pathogenic (*tdh+/trh+*) Vp levels in oysters during ambient storage (28-32°C) for 5 or 24h, then subjected to resubmersion (27-32°C) for two weeks.

Methods: Three 24h storage trials and two 5h storage trials were performed. For each trial, replicate oyster samples were collected at initial harvest (n=2), after ambient air storage (n=2), and after one (n = 4) and two (n = 4) weeks of resubmersion. Concurrently, oysters not ambient stored were collected as a background controls (n = 4). Bacteria levels were measured using an MPN enrichment followed by real-time PCR.

Results: After 5h oyster storage, the mean increase for both organisms was 1.2-1.3 log MPN/g. After 24h storage, the mean Vv and Vp levels increased by 1.5 and 2.3 log MPN/g, respectively. Pathogenic Vp levels increased <0.7 and 2.0 log MPN/g after 5h and 24h storage, respectively. Regardless of storage time, Vv and Vp levels returned to within 0.5 log MPN/g of background levels after one week of resubmersion. After two weeks of resubmersion, total Vv and Vp levels were within 0.1 and 0.4 log MPN/g of background levels, respectively. Pathogenic Vp levels in 24h stored oysters were within 0.5 log MPN/g after two weeks of resubmersion.

Significance: These data demonstrate that resubmersion of oysters exposed to elevated ambient temperatures allowed vibrio levels to return to background levels. These results can inform development of certain oyster aquaculture practices to minimize the risk of Vv and Vp illness.

P3-128 *Vibrio cholerae, V. vulnificus*, and *V. parahaemolyticus* Abundance in Oysters (*Crassostrea virginica*) and Clams (*Mercenaria mercenaria*) from Long Island Sound, U.S

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Introduction: Vibriosis is a leading cause of seafood-associated morbidity and mortality in the United States and internationally. Illness is typically associated with consumption of raw or undercooked oysters, but clam-associated vibriosis is being increasingly reported. However, little is known about the prevalence of *Vibrio* spp. in clams.

Purpose: The study objective was to compare the levels of *V. cholerae* (Vc)*, V. vulnificus* (Vv), and *V. parahaemolyticus* (Vp) in oysters and clams concurrently harvested from Long Island Sound (LIS), USA.

Methods: Shellfish (68 oyster and 30 clam samples) were collected between July 16 and September 24, 2012 following Vp illnesses associated with shellfish from LIS. MPN-real-time PCR methods were used to enumerate of Vc, Vv, and total and pathogenic (*tdh+* and/or *trh+*) Vp.

Results: Total Vc was detected in 8.8% and 3.3% of oyster and clam samples, respectively. In oysters, mean Vc levels in samples above the LOD was 0.20 log MPN/g; the one positive clam sample had 0.48 log MPN/g. *V. vulnificus* was detected in 97% and 90% of oyster and clam samples, with mean levels of 1.3 and 0.10 log MPN/g, respectively. Vp was detected in all samples with mean levels of 2.2 and 1.1 log MPN/g in oysters and clams, respectively. The differences between vibrio levels in the shellfish types was significantly different ($P < 0.001$). However, the differences in detectable levels of pathogenic Vp (*tdh*, $P = 0.329$ and *trh*, $P = 0.292$) in oysters and clams were not significant.

Significance: The data indicate that although levels of total vibrios are higher in oysters than clams, the potentially pathogenic variants are found in similar levels, albeit less frequently. This may explain why vibriosis is associated with clams, when total vibrio levels are lower than in oysters. However, additional data is still needed to ascertain the reason(s) that clams are increasingly associated with vibriosis.

P3-129 High Salinity Relaying to Reduce *V. parahaemolyicus* and *V. vulnificus* in Chesapeake Bay Oysters

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Introduction: Cases of *Vibrio* infections have tripled from 1996 to 2010 and these infections are most often associated with the consumption of *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) contaminated seafood, particularly oysters. Information is inadequate on evaluation and validation of high salinity relaying method to reduce these bacteria in oysters.

Purpose: The purpose of this study was to evaluate and validate a low cost method to reduce the abundance of Vp and Vv in oysters.

Methods: High salinity relaying was carried out in on-shore closed recirculating tanks (RAS) and in the Chesapeake Bay (field) to reduce Vp and Vv numbers in oysters. Moreover, moderate salinity relaying was conducted in the field. Oysters were collected from approved harvest water, temperature abused for 4 hours, then transferred to high (28-34 ppt.) and moderate (13-18 ppt.) salinities. After 7, 14 and 21 days of relaying, oysters were analyzed for Vp and Vv using Multiplex Real Time PCR, and for total coliforms and *Escherichia coli* using petrifilm. Water samples were analyzed for total coliforms, *E .coli*, and total *Vibrio* using membrane filtration procedure.

Results: The counts of Vp and Vv ranged from 3 to 438,399 MPN/g with 2-5 log reductions. The total mortalities of oysters were not higher than 4%. Relaying of oysters to high salinity field sites was found to be more effective in reducing Vv than Vp. In RAS tanks, additional days were required to achieve comparable reductions in Vv and Vp. *E. coli* and total coliforms counts in oyster and water samples ranged from <10 to 18,000 CFU/g and <10 to 340 CFU/100ml, respectively. No concomitant reduction of total coliforms or *E. coli* was observed during relaying trials.

Significance: These data suggest that high salinity relaying of oysters is an effective postharvest mitigation strategy to reduce *Vibrio* in oysters.

P3-130 Modification and Validation of AOAC Official Method 977.13 for Histamine in Seafood to Improve Sample Throughput

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Introduction: Histamine is the main causative agent in scombrotoxin fish poisoning, the most frequently occurring illness related to fish consumption. The current AOAC official method for histamine determination in fish is the fluorometric method (AOAC 977.13), which is sensitive and reproducible, but somewhat labor intensive and time consuming.

Purpose: The objective of this study was to evaluate several promising modifications to the official AOAC fluorometric method with the intention to reduce assay time and increase sample throughput while maintaining the performance of the original method.

Methods: The following modifications to the AOAC method were investigated: replacement of the ion-exchange resin with solid phase extraction (SPE) cartridges; use of pre-treated AG 1-X8 resin; reduction of resin column length from 8 to 4 cm; reduction of sample size from 10 to 5 g; omission of the heating step in the extraction; and replacement of the cuvette style fluorometer with a microplate reader. Yellowfin tuna (*Thunnus albacares*), mahi-mahi (*Coryphaena hippurus*), and Spanish mackerel (*Scomberomorus maculatus*) samples spiked with histamine in concentrations ranging from 5-250 ppm were used to test the modified method. Recovery, precision, and detection limits were evaluated by applying FDA guidelines for single laboratory validation of chemical methods.

Results: Replacing the ion-exchange resin with a SPE cartridge or pre-treated ion-exchange resin, reducing column length of resin from 8 to 4 cm, and reducing sample amount from 10 to 5 g had an adverse effect on the performance characteristics of the method. However, omitting the heating step in the extraction and replacing the cuvette style fluorometer with a microplate reader retained method performance while increasing sample throughput. The recovery, precision (relative standard deviation), and limit of detection of the modified method assessed by the single laboratory validation ranged from 92 to 105%, 1 to 3%, and 0.2 to 0.5 ppm, respectively, in tuna, mahi-mahi, and Spanish mackerel samples.

Significance: We conclude that the AOAC 977.13 fluorometric method, modified as described, will improve assay time and sample throughput efficiency cumulatively, as the number of sample units analyzed increases. This modified method could likely be used by regulatory agency and other laboratories following successful multi-laboratory validation.

P3-131 Optimization and Evaluation of a Regulatory Method for Identifying Crustacean Species Using DNA Barcoding

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Introduction: DNA barcoding involves PCR amplification and sequencing of a short, standardized segment of a gene to discriminate species. FDA has previously validated a regulatory method for identifying vertebrate fish species via barcoding of a standardized ≈655 bp fragment near the 5′ end of the mitochondrial cytochrome *c* oxidase 1 (CO1) gene.

Purpose: This work is part of a project to validate identification of crustacean shellfish using CO1 barcoding.

Methods: A ≈655 bp fragment of the CO1 gene was amplified using PCR, and products were sequenced in both forward and reverse directions using the Sanger method. Sequences were aligned and used to generate neighbor-joining consensus trees. In addition to assessing intra- and inter-species variation versus sequencing (run to run) variation, we optimized PCR and determined the effects of sample handling and storage.

Results: Adverse sample handling, such as multiple freeze-thaw cycles, were found to adversely affect CO1 PCR results for some products. This is in contrast to previous experience with vertebrate fish tissues. In nearly all tests of inter- and intra-species variation, we obtained bidirectional sequence reads at least 650 bp in length with 98-100% high quality base calls. Results indicate that the method is able to effectively differentiate most commercial crustacean species and successfully identify unknowns. A library of reference standard COI sequences is also being developed.

Significance: DNA barcoding has proven to be an accurate and reliable method for species identification of processed and packaged seafood which cannot be identified through standard morphological means. Species identification is important for both protecting food safety and detecting seafood fraud. Our work indicates that CO1 DNA barcoding, previously proven to be a useful tool for the identification of vertebrate fish species, will also be a valuable tool for identifying commercial crustacean shellfish.

P3-132 Minimizing *Salmonella* Attachment to Dry Surfaces through Use of High-frequency Mechanical Vibration

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Introduction: Low-moisture foods are prone to cross-contamination with *Salmonella* during processing. However, since wet sanitation procedures cannot be used in dry processing facilities, alternative dry sanitation practices must be developed to minimize microbial cross-contamination.

Purpose: This study aimed to assess the efficacy of high-frequency mechanical vibration for dislodging *Salmonella* and *Enterococcus faecium* from processing surfaces under controlled environmental conditions.

Methods: Wheat flour was inoculated with *Salmonella* Enteritidis PT30 or *E. faecium* using a glass bead dry transfer method to achieve populations of 6.7 and 6.9 log CFU/g, respectively. After conditioning to an a_w of 0.45 in an environmental chamber, the inoculated powder was applied to stainless steel coupons using an electrostatic powder coating gun. The coupons were then treated with two vibration modes and frequencies (28 and 40 kHz) using piezoelectric transducers, then plated on modified TSA or MRS media to enumerate *Salmonella* and *E. faecium*, respectively. Also, surfaces were analyzed via scanning electron microscopy.

Results: Populations of *Salmonella* and *E. faecium* in the inoculated wheat flour gradually decreased to 6.2 and 4.2 log CFU/g, respectively, after 10 d of conditioning at 45% RH. The electrostatic attachment method yielded significantly ($P < 0.05$) greater surface attachment for *Salmonella* (39.1 CFU/cm^2) than *E. faecium* (1.2 CFU/cm^2), with significant variations in cell density also observed across the replications. However, HFNA treatment was able to reduce *Salmonella* populations on the stainless steel coupons by ~69 and 66% at 28 and 40 kHz, respectively, which was confirmed with variable pressure electron microscopy.

Significance: The findings will contribute to understanding the physical characteristics of bacterial attachment to dry surfaces and the potential efficacy of high-frequency mechanical vibration for dry equipment decontamination.

P3-133 In-plant Validation of Pet Food Processing for the Elimination of Pathogens

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Introduction: Pet food companies are coming under increased scrutiny due to recent recalls. Additionally, the Food Safety Modernization Act has recently proposed regulations for pet food producers that include a requirement for risk-based preventive controls. Therefore, it is important to validate production processes for the elimination of pathogens in pet food.

Purpose: The objective of this study was to utilize non-pathogenic *E. coli* strains as surrogates for *E. coli* O157:H7 to perform an in-plant validation for multiple pet food formulations.

Methods: Six *E.* coli strains were selected as surrogates and combined in equal portions to create 10^9 CFU/ml culture cocktails to inoculate four 907 kg batches of pet food. For each batch, 1 l of cocktail was added in the initial mixing step. Samples were collected after completion of mixing, post-extrusion, and post-drying for each batch. Samples were enumerated with 3 M Coliform/*E. coli* petrifilm. Surface swabs were taken following post-production equipment cleaning and sanitizing and analyzed for the presence of *Salmonella* using the BAX system.

Results: The samples from the mixer resulted in a 10^3 to 10^4 CFU/g inoculation level. The temperature of the extruded pet food reached 117.2°C, 91°C, 101.7°C, and 107.8°C for pet food batches 1, 2, 3, and 4, respectively. After extrusion, no growth was detected across all batches. Pet food batches 1 and 2 were combined in the drying step; likewise, batches 3 and 4 were combined with all post-drying samples showing no growth. Post-sanitation swabs taken of the equipment were negative for *Salmonella.*

Significance: This in-plant validation of pet food processing demonstrates the use of non-pathogenic surrogate organisms that have been selected for their behavior which mimics their pathogenic counterparts. Validations for processing and the destruction of pathogens are an important practice to fulfill the more rigorous requirements being developed for pet food regulations.

P3-134 Combined Treatment of Slightly Acidic Electrolyzed Water and Fumaric Acid to Improve Microbial Safety and Quality of Fresh Pork

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Introduction: Pathogens contamination in fresh pork such as *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*, has mainly impacted its safety and quality. Therefore, an effective sanitizing treatment to improve the microbial safety and quality of pork during processing and storage is necessary.

Purpose: This study was conducted to compare the effectiveness of individual treatments (slightly acidic electrolyzed water [SAcEW] and fumaric acid [FA]) and their combinations on reducing *S.* Typhimurium, *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* in fresh pork, as well as to study the shelf life of pork based on microbial and sensorial quality during storage at 4 and 10°C.

Methods: Pork samples were immersed in various single treatments with or without mild heat (40°C) for 3 min. For the efficacy of combination treatments, samples were treated with the SAcEW (30 mg/l ACC) and 0.5% FA either sequentially or simultaneously, and with or without mild heat.

Results: The decontamination of fresh pork by SAcEW and 0.5% FA at 40°C for 3 min (SAcEW + 0.5% FA) significantly ($P < 0.05$) reduced the initial bacterial loads and retarded bacterial growth during storage at 4 and 10°C. The reductions of aforementioned pathogens (2.38 – 2.99 log CFU/g) were achieved with SAcEW + 0.5% FA and significantly different ($P< 0.05$) compared to those of other treatments. Based on microbiological and sensorial point of view, this combined treatment also prolonged the shelf life of pork up to 6 days when stored at 4°C, whilst 4–5 days when stored at 10°C, compared to that of control.

Significance: The combined treatment of SAcEW + 0.5% FA seems to be a very promising method to ensure microbial safety and quality of pork, which may benefit to the pork industries.

P3-135 Effects of Different Sanitizers on the Toxin Production of Non-O157 Shiga Toxin-producing *Escherichia coli* Serotypes

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Introduction: Foodborne outbreaks caused by non-O157 Shiga toxin-producing *Escherichia coli* (nSTEC) has increased in recent years. STEC strains can cause diarrhea, hemorrhagic colitis and in some severe cases potentially life threatening haemolytic uremic syndrome (HUS). Toxins produced by nSTEC are critical virulence factor that contribute to the intestinal epithelia damage and to the development of HUS.

Purpose: Investigate the effect of different common chemical decontamination systems on nSTEC toxin production using a quantitative microtiter cytotoxicity assay.

Methods: Shiga toxin-producing *E. coli* serotypes O26, O103, O45, O111, O121 and O145 (nSTEC) were used in this study. Toxins were partially purified and exposed to different stress (100 ppm peroaxyacetic acid, 25% octanoic acid, 4% lactic acid and 100 ppm sodium hypochlorite solutions). After neutralization, toxin serial dilutions were added to Vero cell cultures. A staining procedure was used to evaluate cells attachment to monolayers and consequently the toxins potency. Cytotoxic effects were quantitatively measured by a spectrophotometer.

Results: Cell detachment varied as a function of cell concentration, incubation time and stress agent. Significant difference ($P < 0.05$) between control and stress conditions were observed when toxins were treated with peroaxyacetic acid and sodium hyphochlorite solutions, while no significant effects were observed ($P > 0.05$) with octanoic acid and lactic acid solutions.

Significance: Findings of this study could be useful to direct future antimicrobial strategies in the food industry.

P3-136 Inactivation of *Escherichia coli* O157:H7 from Pak Choi Seeds Using Sequential Treatments of Chlorine Dioxide, Drying, and Dry-heat

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Developing Scientist Competition

Introduction: Vegetable seed sprouts have a high risk of food poisoning because they are frequently consumed raw. Even if sprout seeds are contaminated with low levels of foodborne pathogens, the population of pathogens can be increased to high levels during cultivation of the seeds. Therefore, it is important to eliminate foodborne pathogens from sprout seeds.

Purpose: The objectives of this study were to screen the seeds that are resistant to aqueous chlorine dioxide (ClO_2) and to develop a method to eliminate *Escherichia coli* O157:H7 from the seeds.

Methods: To screen seeds resistant to ClO_2, 15 types of sprouts seeds were treated with ClO_2 (200 µg/ml) solution for 5 min and then their germination rates were evaluated. To eliminate *E. coli* O157:H7 from sprout seeds, seeds were treated with ClO_2 (0, 50, or 200 µg/ml) for 5 min, dried at 45°C with 23% relative humidity (RH) for 24h, and heated at 80°C with 23% RH for up to 24h. After the treatments, the population of *E. coli* O157:H7 on the seeds and the germination rate were measured.

Poster

Results: Pak Choi seeds showed the highest germination rate among 15 types of seeds after treatment with ClO_2. When Pak Choi seeds containing *E. coli* O157:H7 (6 log CFU/g) were treated with 0, 50, and 200 µg/ml ClO_2 for 5 min, the numbers of *E. coli* O157:H7 decreased to 5.0, 2.4, and 1.0 log CFU/g, respectively. When those seeds were dried and dry-heated, *E. coli* O157:H7 were completely inactivated from the seeds and the germination rate was 89%.

Significance: This study showed that *E. coli* O157:H7 on Pak Choi seeds were completely inactivated by sequential treatment of ClO_2, drying, and dry-heat without decreasing the germination rate.

P3-137 A Benchtop Biofilm Test System to Benchmark Sanitation Performance and Efficacy

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Introduction: developed a small scale, bench type biofilm reactor to create challenge replicates of various biofilm strains of microbes. Objective was to evaluate a variety of cleaners and sanitizers combinations to benchmark efficacy on conveyor belt type HDPE surfaces.

Purpose: This study is to evaluate the removal of mixed *P. fluorescens*, *L. monocytogenes*, and *S. typhimurium* based biofilms on industrial food processing plant equipment by evaluating the efficacy of foam alkaline, chlorinated alkaline, neutral foam cleaners, and acidic foam cleaners. This was followed by either a sodium hypochlorite, chlorine dioxide, quaternary ammonia, or peroxyacetic acid sanitizer exposure.

Methods: Bacterial cultures were inoculated into dynamic low nutrient flow biofilm generator where biofilms formed and matured on suspended High Density Polyethylene (HDPE) coupons for 72h. Once the biofilms were established, the HDPE coupons were removed and exposed to a water rinse to remove any planktonic cells. The rinse was followed by various foam cleaner and sanitizer combinations. Following sanitization, coupons were fully neutralized in D/E broth and vortexed to remove the remaining viable sessile cells. Serial dilutions were plated onto Tryptone Soy Fast Green (TSAF) and compared against control coupons to determine log reductions.

Results: The experimental combinations tested to date, show the chlorinated alkaline cleaner with chlorine sanitizer combination having the highest reduction 6.63 log CFU/cm^2, followed by the mild non-chlorinated alkaline cleaner followed by peroxyacetic acid at 4.98 log CFU/cm^2. The least efficacious combination to date was the mild alkaline cleaner followed by the quaternary ammonia sanitizer at 1.64 log CFU/cm^2.

Significance: The ability to replicate an immature or mature biofilm enables the experimenter to test a wide variety of cleaner-sanitizer combinations utilized in the removal of typical food plant biofilms. This enables one to create a functional database of the variety of cleaner and sanitizer combinations vs a variety of meat [in this case], dairy, and produce biofilms. It also has application for other food market plant biofilms as well.

P3-138 Assessing the Variability in Biofilm Formation by Multiple *Listeria monocytogenes* Strains and Impact of Contact Surfaces on Susceptibility to Disinfectants

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* survival and persistence on food equipment surfaces poses a severe safety risk and has drawn attention due to unclear evidence of mechanisms implemented in resistance to sanitizers and food contamination phenomena.

Purpose: (i) To investigate the biofilm formation potential of strains of different origin and lineages under various environmental conditions and (ii) to correlate biofilm formation with resistant mechanisms of the pathogen to biocides.

Methods: Twenty-three strains from various food processing equipment and food products were evaluated for attachment potential on polystyrene microplates in Tryptic Soy Broth (TSB) or TSB/10, after 72h at 20 or 37°C. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations were evaluated for peroxyacetic acid (PAA) and quaternary ammonium compounds (QACs). Hydrophobicity of cell surface, and comparative resistance of biofilms, formed on stainless still and polystyrene, to 2000 ppm PAA and 500 ppm QACs for 5 min were evaluated. Experiments were performed in three technical and three biological replicates.

Results: Results revealed that biofilm formation was correlated with strain origin, environmental conditions and cell surface hydrophobicity. Equipment isolates were able to attach in higher populations to polystyrene in contrast to food isolates, and high electron donating nature contributed to higher attachment; no significant lineage effect was observed ($P > 0.05$). Strongly adherent strains were markedly influenced by growth conditions unlike weakly or moderately adherent strains, while exhibited the lowest MIC and MBC to PAA. Food isolates demonstrated higher resistance to biocides compared to equipment isolates. QACs and PAA effectiveness against biofilm cells were observed to increase on polystyrene and stainless steel, respectively, and both biocides were significantly more effective against biofilms formed on stainless still than those attached to polystyrene surface ($P < 0.05$).

Significance: Clarification of multivariate interactions between acquired and endogenous characteristics of *L. monocytogenes* may lead to design more accurate preventing systems to safety directions.

P3-139 Inhibition of *Listeria monocytogenes* from Ready-to-Eat Foods and Food Processing Environments in British Columbia by Quaternary Ammonium Based Biocides and Triclosan

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Developing Scientist Competition

Introduction: *Listeria monocytogenes* is a foodborne pathogen, causing severe disease and mortality (20-40%) in susceptible populations. *L. monocytogenes* is a significant problem to the ready-to-eat (RTE) food industry due to its ability to persist in food processing environments (FPEs). Frequent use of sanitizers in FPEs is concerning, as it may select for resistant *L. monocytogenes*. Biocide resistance may facilitate persistence among some strains of *L. monocytogenes* and increase the risk of product contamination.

Purpose: To assess the resistance of *L. monocytogenes* from RTE foods and FPEs to common biocides.

Methods: The minimum inhibitory concentration (MIC) of benzalkonium chloride (BAC), E-san (ES), and triclosan (TRI) was determined for *L. monocytogenes* (n = 42) isolates from RTE foods or RTE FPEs. Isolates were grown overnight on blood agar plates at 37°C and one colony was suspended in Mueller-Hinton broth. Aliquots were spotted onto Mueller-Hinton agar with 2% sheep blood containing appropriate concentrations of biocide. Growth was examined following incubation at 37°C for 24-48 hours. An established resistance breakpoint (10µg/ml) was used for all biocides. BAC and ES resistant isolates were PCR screened for *bcrABC*.

Results: Resistance to BAC between Lineage I and Lineage II isolates (4.3% and 51.5%, respectively) did differ significantly ($P = 0.00014$). Differences ($P < 0.001$) between lineages existed for TRI, with 95.7% of Lineage I isolates and 39.3% of Lineage II isolates being resistant. Lineage I and Lineage II isolates differed ($P = 0.00014$) in resistance to ES; specifically, 4.3% and 51.5% of Lineage I and Lineage II isolates, respectively, displayed resistance. Eighteen isolates had high MICs for BAC and ES (20-25µg/ml); fifteen of these were positive for *bcrABC*, all of which were Lineage II.

Significance: While MICs remain below recommended concentrations for these biocides, isolates with increased resistance to sanitizers are a cause for concern as it may contribute to persistence in the FPE.

P3-140 An Evaluation of HiCap™ Neutralizing Broth for Detection of *Listeria* spp. from Environmental Sampling Sponges Dosed with Three Different Types of Sanitizers

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Introduction: HiCap™ Neutralizing Broth (HC) is intended for use with sponges and swabs for environmental monitoring programs. HC is formulated to have high capacity to neutralize sanitizers, to be compatible with pathogen detection kits, and to be acceptable for use in production areas including food contact surfaces.

Purpose: The purpose of this study was to compare recovery of *Listeria* inoculated onto sponges hydrated with HC or neutralizing buffer (NB) dosed with low or high levels of sanitizer.

Methods: Polyurethane sponges pre-hydrated with 10 ml of HC or NB were dosed with low level (0.6 - 0.8 mg) or high level (3 mg) of a quaternary ammonium, chlorine, or mixed acid sanitizer. The sponges were inoculated with approximately 800 cells of a *Listeria* cocktail (containing two strains of *L. monocytogenes* and 1 strain each of *L. innocua* and *L. ivanovii)*, placed into refrigeration and shipped to 9 participating laboratories. Detection was performed 72 hours after inoculation using commercial immunodiagnostic or molecular amplification kits.

Results: *Listeria*-positive results were obtained with all 81 sponges hydrated with HC and dosed with no, low or high levels of sanitizer. For sponges hydrated with NB, *Listeria*-positive results were obtained with all 27 sponges receiving no sanitizer, 22 out of 27 sponges receiving low level, and 16 out of 27 sponges receiving high level of sanitizer. NB sponges dosed with low and high levels of chlorine or mixed acid sanitizer produced the *Listeria*-negative results.

Significance: Sampling devices used in environmental monitoring programs must maintain the viability of target organisms until samples are processed in the laboratory, even if residual sanitizer is present. HC was better than NB at maintaining the viability of *Listeria* for 72 hours at refrigerated temperatures in the presence of chlorine and mixed acid sanitizers.

P3-141 Inactivation of *Bacillus cereus* Attached to and in Biofilm on a Stainless Steel Surface by Combined Treatment of Gaseous Chlorine Dioxide and Heat

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Developing Scientist Competition

Introduction: Cross-contamination of foodborne pathogens is one of the most common causes of foodborne illnesses. To prevent the cross-contamination, foodborne pathogens should be effectively decontaminated from food-contact surface and the sanitizers used should not be remained on the surface.

Purpose: This study was done to evaluate the lethal activity of gaseous ClO_2 and its synergistic lethal effect with heat treatment against *B. cereus* cells attached and in biofilm on stainless steel surface.

Methods: Spores of *B. cereus* were prepared and attached on stainless steel coupons (SSCs). Biofilm of *B. cereus* was formed on SSCs in tryptic soy broth at 22°C for 24h followed by exposing to atmosphere with 100 % relative humidity at 22°C for 4 days to induce the sporulation. SSCs containing *B. cereus* (attached spores or cells in biofilm) were incubated with gaseous ClO_2 (115-120 µg/ml) in an airtight container at 25°C or 55°C for up to 6h and the numbers of viable cells of *B. cereus* on SSCs were measured.

Results: When treated with gaseous ClO_2, *B. cereus* spore (5.3 log CFU/coupon) attached to SSC was completely inactivated within 1h and 30 min at 25°C and 55°C, respectively. *B. cereus* (5.9 log CFU/coupon) in biofilm on SSCs was completely inactivated within 6h regardless of storage temperature after exposed to gaseous ClO_2.

Significance: We confirmed that ClO_2 gas was effective in killing *B. cereus* spores attached to or cells in biofilm on SSCs. The synergistic lethal effect between ClO_2 gas and heat treatments was also observed.

P3-142 Effect of Delmopinol Hydrochloride on the Prevention and Removal of *Listeria monocytogenes* and *Salmonella enterica* Stainless Steel-adhered Biofilms

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Introduction: Bacterial biofilms attached to food contact surfaces are an ongoing concern for the food industry due to the resistance of bacteria within biofilms to detergents and sanitizers.

Purpose: To determine the effect of pre-and post-exposure of delmopinol hydrochloride on the prevention and removal of *Listeria monocytogenes* 1/2a and *S. enterica* Agona biofilms on stainless steel.

Methods: Biofilm formation - Stainless steel blanks (2cm x 2cm) were spot-inoculated on the finished surface with 20µl of a 10^9 CFU/ml liquid culture, placed inoculated side down onto TSA, and incubated for 24h at 25°C. Following biofilm formation, blanks were rinsed with phosphate buffered saline (PBS), vortexed for 90s in PBS, and surface-plated onto TSA for enumeration. In pre-exposure studies, the blanks were submerged in delmopinol hydrochloride solution (0.2% and 0.5%) for 1, 5, and 10 min prior to biofilm formation. In post-exposure studies, the blanks received no pretreatment with delmopinol but were exposed to the solution as above after biofilm formation.

Results: Pre-exposure of the blanks to 0.2% delmopinol resulted in a significant decrease in *L. monocytogenes* concentration at 1, 5 and 10 min exposures ($P < 0.05$). However, pre-exposures with the 0.5% solution had no significant effect on *L. monocytogenes* biofilm populations ($P > 0.05$). All post-exposures lead to a significant decline in biofilm concentrations ($P < 0.05$). Post-exposures of 10 minutes exhibited a mean log reduction of 5.59 and 6.40 for 0.2% and 0.5% delmopinol solutions, respectively. For *S.* Agona, pre-exposure resulted in minimal reductions in bacterial recovery ($P < 0.05$). Whereas, post-exposures of 10 min exhibited a mean log reductions of 7.65 and 7.75 for 0.2% and 0.5% delmopinol solutions, respectively.

Poster

Significance: Delmopinol hydrochloride was significantly better for removing *L. monocytogenes* and *S. Agona* biofilms on stainless steel than preventing them.

P3-143 Efficacy of Sanitizers to *Bacillus cereus* Biofilm on Food Contact Surfaces

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Introduction: Biofilms in which the spore-forming *Bacillus cereus* readily forms on various types of food contact surfaces are resistant to sanitizers. The effectiveness of sanitizers for *B. cereus* biofilms depends on the surface material and the condition with regards to whether a scratch exists or not.

Purpose: This study was conducted to compare the 4 sanitizers' effectiveness against *B. cereus* biofilms on 5 different surface materials and surface conditions such as being scratched or non-scratched.

Methods: Five coupons (glass, GL; stainless steel, SUS; polyethylene, PE; wood) were made to 10 (2 by 5) cm^2 and were prepared as either scratched or non-scratched. All coupons were immersed in spore suspensions (27°C for 4h) and were incubated in the RH 100% tube to form biofilms (27°C for 4 days). Each coupon on the biofilms was treated with chlorine (Cl), chlorine dioxide (ClO_2), quaternary ammonium compound (QAC), and alcohol of 70% solution (Cl, ClO_2, QAC, 200ppm).

Results: The SUS indicated the lowest rate of adherence (6.81 ± 0.05 log CFU/10 cm^2). The wood showed lowest effects of sterilization for all conditions. Cl was proven to be the most effective for controlling *B. cereus* biofilms on most of the coupons. Sterilization efficacy on the scratched SUS was lower than the non-scratched SUS by 5.95 ± 1.13 and 5.57 ± 0.21 log CFU/10 cm^2, respectively ($P < 0.05$).

Significance: The types of surface materials should be considered when making purchases by food service operations. In addition, a guideline for the prevention of scratches on the surface food materials and the concentration levels of sanitizers based on the condition of scratched materials is necessary.

P3-144 Effects of Disinfectants on Decontamination of *Salmonella* Newport in Irrigation Well and Pond Water

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Introduction: More than 20% of outbreaks of foodborne diseases have been traced back to contaminated fresh produce. Pre-harvest has been proposed to be one of the most likely points of original contamination, and irrigation water has been reported as one of the main contamination sources. However, little is known about the efficient methods for the decontamination of *Salmonella enterica* in irrigation water.

Purpose: To evaluate the efficacy of disinfectants, Clorox Regular-Bleach (sodium hypochlorite), Oxidate Broad Spectrum Bactericide Fungicide (hydrogen peroxide) and Sanidate 12.0 (hydrogen peroxide and peroxyacetic acid), on the reduction of *S. enterica* serovar Newport in irrigation water.

Methods: In this study, well and pond irrigation water were collected from different vegetable farms on Eastern Shore of Virginia. Three initial levels of *Salmonella* Newport (10^8, 10^6 and 10^4 CFU/ml) and two application levels of disinfectants (1:1,000 or 1:50,000 dilution) were tested in this study. Bacterial concentration was measured 30 min after treatment.

Results: *Salmonella* Newport in irrigation well and pond water was not detectable after application of the three disinfectants tested in this study at the initial bacterial level of 10^4 CFU/ml. Sanidate 12.0 is the most efficient disinfectant for water treatment with high *Salmonella* concentration. The efficacy of the disinfectants was lower for pond water treatment than that for well water treatment. Water pH was changed after the disinfectant treatments especially applied with higher concentration of sanitizers.

Significance: These results would bring clues to manage *Salmonella* contamination in irrigation water for vegetable and fruit production.

P3-145 Development of a Standardized Method for Norovirus Virucidal Testing on Soft Surfaces

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Introduction: Human Noroviruses (HuNoV) are a leading cause of acute gastroenteritis worldwide. HuNoV can persist in the environment making preventing HuNoV infections and controlling outbreaks extremely challenging. Epidemiological evidence suggests that soft surfaces may be a relevant source of HuNoV due to ineffective decontamination.

Purpose: The aim of this study was to develop a protocol to test the efficacy of disinfectants against HuNoV on soft surfaces.

Methods: Feline Calicivirus (FCV) strain F9 and Murine Norovirus (MNV) strain 1 were dried onto 1x1 in coupons of glass, cotton, and polyester and recovered using sonication for 5 min at 40 kHz and stomaching for 5 min at 260 rpm. For disinfection viruses were inoculated and dried in the same manner and subjected to disinfection with 5,000 ppm Clorox or 2,6000 ppm Virox for 5 min followed by neutralization in PBS+0.02%Tween80+10%FBS. Viruses were enumerated using plaque assay and confirmed via reverse-transcriptase-PCR.

Results: Recovery efficiency from glass, polyester, and cotton was 35, 6, and 0.15% for FCV, and 9.2, 3.1, and 0.57% for MNV, respectively. Longer drying time (40 min) resulted in less recovery of viruses from soft surfaces. Clorox (5,000 ppm) and Virox (ca. 2,600 ppm) inactivated FCV below the limit of detection (1.34 PFU) corresponding to inactivation rates of >99.99, >99.9, and >90% for glass, polyester, and cotton, respectively.

Significance: Our results indicate virus recovery can be affected by virus type, surface characteristics, and attachment time. Due to the low recovery rates associated with cotton, it will be especially challenging to demonstrate complete 3 log inactivation (>99.9%) of viruses on soft surfaces.

P3-146 Efficacy of Ethanol-based Sanitizer Containing Persimmon Extract on Norovirus and its Surrogate, Bacteriophage MS2

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Introduction: Human noroviruses (NoVs) are the leading causative agents of gastroenteritis and foodborne illnesses worldwide. Disinfection is an essential approach to the prevention of NoV infections. We found that persimmon extract (PE) had a strong effect on NoVs, and successfully developed an antinoroviral ethanol-based sanitizer containing PE (NA-20) and food additives.

Purpose: The objective in this study was to evaluate the antiviral effects of NA-20 on NoV GII.4 and MS2 phage, a surrogate of norovirus, and to identify the mechanism of action of NA-20.

Methods: The antiviral efficacy of four solutions (NA-20, NA-20 without PE, 50% ethanol, and PBS as the control) against NoVs was evaluated by quantifying the amount of residual noroviral genome using quantitative real-time RT–PCR. The antiviral efficacy against MS2 phage was examined with

an infectivity assay (plaque assay) and quantification of the residual genome. The effects of the four solutions on the morphology of MS2 phage were assessed with transmission electron microscopy.

Results: The amount of residual viral genome of both viruses decreased significantly when treated with NA-20 ($P < 0.05$). NA-20 inactivated MS2 phage by 3.06 log PFU/ml, whereas NA-20 without PE and 50% EtOH did not reduce the infectivity of MS2 phage (0.03 and 0.04 log reduction, respectively). MS2 phage particles were denatured and aggregated by the NA-20 treatment.

Significance: These results indicate that the reduction of both viral genomes and the inactivation of MS2 phage were caused by the astringency of the PE in NA-20. This suggests that NA-20 is useful for the disinfection of NoVs in the kitchen and food industry because it is harmless and can be used with cookware and hands.

P3-147 Assessment of Food Safety and Sanitation Risks in the Kitchens of Consumers in an Urban Environment

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Introduction: Research utilizing both survey and observational techniques has found that consumer reporting of their own safe food handling habits may not be completely reliable. Previous research in our laboratory has resulted in the development, piloting and publication of an audit tool for researchers to objectively assess food safety risks and sanitation in the domestic kitchen. This tool allows trained researchers to observe and document potential food safety risks in the kitchens of consumers.

Purpose: The objective of the research reported here was to systematically and objectively observe and document conditions related to food safety risks and sanitation in the kitchens of 100 consumers in an urban environment.

Methods: Participants were recruited from the city of Philadelphia, PA, utilizing flyers combined with a screening phone call. Homes were visited by two trained researchers who independently utilized the previously developed audit tool to document conditions related to sanitation, refrigeration and food storage in the homes of 100 consumers. Consumers were reimbursed $50 cash for their participation. **Results** of the audit tool observations were used to measure consumers' compliance with recommended food safety practices.

Results: Participants demographics included 47% African American, 39% Caucasian and 12% Hispanic. Forty-four percent of participants had a household income below $25,000/year. Potential food safety risks identified included evidence of pest infestation in kitchens (68%), perishable food stored at room temperature (28%), and raw meat stored above ready-to-eat foods or packaged incorrectly (97% of homes where raw meat was present). Only 4% of refrigerators had a thermometer, 42% of refrigerators were above 5°C and 3% of homes did not have hot water.

Significance: This research indicates that significant food safety and sanitation risks, including high levels of pest infestation, may exist in the homes of urban consumers.

P3-148 Efficacy of Warewashing Protocols and Selected Sanitizers for Removal of Foodborne Bacteria and Viruses from Contaminated Tableware

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Introduction: Epidemiological outbreak data have identified five major risk factors related to foodborne outbreaks in retail food establishments. The U.S. FDA Food Code addresses controls for all five, including science-based controls for contaminated equipment. One limitation of the controls related to contaminated equipment is that they only address how to reduce bacteria on food-contact surfaces and not viral agents.

Purpose: The purpose of this study was to evaluate the efficacy of selected sanitizers (tap water control, 200 ppm sodium hypochlorite, acidic electrolyzed water [AEW] and neutral electrolyzed water [NEW]) for removal of bacteria (*Salmonella* Typhimurium, *Listeria innocua*) and human norovirus surrogates (murine norovirus [MNV], Tulane virus [TV]) from contaminated tableware using established manual and mechanical warewashing protocols.

Methods: Cream cheese was inoculated with *L. innocua, S.* Typhimurium, MNV, or TV. The cheese was applied to ceramic plates to simulate a worst-case contamination scenario. Plates were cleaned using manual (43°C) and mechanical (49°C) warewashing protocols, and sanitized with one of the test sanitizers. After 1 hour of air-drying, plate surfaces were swabbed and surviving bacteria quantified using nonselective and selective agars, while viruses were quantified using viral plaque assays.

Results: Manual and mechanical warewashing procedures produced a > 5 log and < 4 log reduction in *S.* Typhimurium and *L. innocua*, respectively. MNV and TV showed a < 4 log reduction in viral titer after warewashing. The use of 200 ppm sodium hypochlorite, AEW, or NEW sanitizers only slightly enhanced microbial inactivation when compared to a tap water control.

Significance: These data suggest that current manual and mechanical warewashing operations are sufficient for removal of bacteria from contaminated tableware, but are not as effective on foodborne viruses. Since noroviruses are highly infectious, optimizing warewashing procedures to remove these pathogens is a significant public health challenge.

P3-149 Small Flies as Vectors of Foodborne Illness: Cross-contamination of Food with *Escherichia coli* by Indoor Breeding Fruit Flies

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Introduction: More than 50% of food handling establishments in the US experience fruit fly (*Drosophila* spp.) infestations. Little is known about their potential to transfer foodborne and other pathogen from their breeding site to food and food handling surfaces.

Purpose: This research presents results from comprehensive studies of the capability of fruit flies to transfer bacteria in the laboratory. The aim of this study was to provide evidence of the ability of small flies to act as vectors for cross contamination in foods.

Methods: Up to 20 fruit flies were introduced to a 1 ft^3 enclosure containing the following: 10g of sanitized chopped lettuce and two 10g samples of yeast-risen donut. One of the donut samples was previously inoculated with >1 x 10^6 CFU/g Nalidixic acid-resistant *E. coli.* A control box contained similar samples without *E. coli.* Following a two day exposure of flies to food samples the flies were exterminated and samples were enumerated for *E. coli* using MacConkey agar supplemented with Nalidixic Acid. Scanning electron microscopy was used to visualize contaminated flies.

Results: Over the course of three replicate experiments cross contamination of food with *E. coli* occurred in both foods during the first replicate and in either lettuce or donut during the other two. *E. coli* was found at levels of 1.2 to 1.9 x 10^3 CFU/g in the non-inoculated donut samples and 1.1 to 4.20 x 10^3 CFU/g in the non-inoculated lettuce sample. Bacteria can be seen on the appendages in SEMs of contaminated flies. *E. coli* was not detected in control samples.

Significance: Recovery of *E. coli* from non-inoculated food samples that have been exposed to fruit flies with access to contaminated food proves that fruit flies can be effective vectors of foodborne illness and should be considered as a risk to food safety in kitchen and food manufacture environments.

P3-150 An Investigation of Tailgaters' Food-Handling Behaviors

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Introduction: Previous research indicates that unsafe food-handling behaviors are common among consumers. Given the setting of most tailgating events, individuals participating in such activities may be at an increased risk for foodborne illnesses.

Purpose: The purpose of this study was to investigate tailgaters' food-handling behaviors.

Methods: Participants completed a written food safety questionnaire which included subscales previously developed and validated using food safety expert input, pilot study feedback, measures of internal consistency, and principal components analysis. Food-handling behaviors were measured using the Cross Contamination Subscale (5 items) and the Sanitation Subscale (3 items). Perceived threat was measured using the Perceived Severity of Foodborne Illness Subscale and the Perceived Susceptibility to Foodborne Illness Subscale. The Susceptibility Subscale (3 items) measured beliefs related to one's own risk of developing a foodborne illness while the Severity Subscale (5 items) measured beliefs regarding the seriousness of the consequences that could occur should one develop a foodborne illness. Higher food-handling scores and higher perceived threat scores indicated safer food-handling and greater threat respectively. Spearman rank correlation coefficients and *t*-tests were used to measure the strength of the association between variables.

Results: A total of 125 completed surveys were collected from eligible participants who are 18 years old and above and participated in a college football tailgating activity. The age of participants was 32.31 ± 16.41. Most were male (54.4%), white (81.3%), and completed high school or beyond (83.0%). Cross Contamination Subscale scores were positively associated with Sanitation Subscale (p=.37, P< 0.01) and Severity Subscale scores (p=.32, $P < 0.01$). Women (10.29 ± 1.66; 28.66 ± 3.46) scored significantly higher than men (9.46 ± 1.96; 27.37±3.76) on the Sanitation Subscales ($P < 0.05$) and perceived threat ($P < 0.05$), respectively.

Significance: Findings contribute to the understanding of tailgaters' food-handling behaviors and could help food safety professionals develop effective education programs.

P3-151 Evaluation of Food Safety Knowledge and Practices among Campers in the U.S

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Poster

Introduction: Among the 6,674 foodborne outbreaks (FBO) reported between 1999 and 2008, 141 were associated with food served at camp sites/picnic areas/farms, involving 4,849 individual illnesses.

Purpose: The purpose of this study is to evaluate the level of food safety knowledge and practices among campers in the U.S.

Methods: Data collected by a market research company allowed researchers to recruit respondents across the U.S. The survey instrument was developed based on the literature review and face-to-face interviews; it was validated and pilot-tested prior to data collection. The variables were food safety knowledge (i.e., cross-contamination, hygiene practices, food cooking temperature, and common sources of FBO pathogens) and food safety practices (1 being "almost never"; 5 being "always"). Descriptive statistics for the data summary and inferential statistics like Pearson chi-square and *t*-tests were conducted to explore relationships among variables.

Results: A total of 338 surveys were collected from participants—18 years and above—who were interested in camping/hiking/outdoor activities (93.4%). Only 286 surveys were deemed usable for data analysis. The participants' ages were 61.5 ± 13.6. Most were female (66.8%), white (95.8%), held bachelor degrees or above (65.3%), had no work experience in foodservice operations (75.1%), and had never undergone food safety training (85.7%). Participants received the highest score for the knowledge questions of safe cooking times/cooking temperatures/food storage (70.8%), followed by cross-contamination prevention (60%), groups at greatest risk for FBO (55%), common sources of FBO (48.8%), and foods that increase risk of FBO (43.3%). Overall, food safety knowledge was significantly associated with education ($P < 0.01$), food safety training experience ($P < 0.05$), and practices (3.6 ± 0.4) ($P < 0.01$).

Significance: Results will aid public awareness regarding the food safety risks associated with outdoor activities and may help food safety educators better understand U.S. campers' food safety behaviors.

P3-152 Restaurants' Preparedness for Food Allergies

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Introduction: Restaurants serve 70 billion meals annually with 130 million Americans dining out daily. Of this figure, approximately 10% of customers suffer from various forms of food allergies. Providing allergen-free food is challenging as most food allergic reactions occur in restaurants.

Purpose: The purpose of this study was to investigate restaurant operators' food allergy knowledge, training procedures, and in-store policies related to serving clients with food allergies.

Methods: The questionnaire was developed based on interviews with three restaurant owners and literature review. Research panels consisted of restaurant owners and managers in the U.S. were recruited by a professional marketing research company.

Results: A total of 110 participants completed this survey. About 79% (n = 87) participants had never received any food allergy training. Of maximum 28 points possible, the mean food allergy knowledge score was 19.7 ± 4.6 (range = 6 to 27). Only 69% (n = 76) respondents had provided food allergy training to their employees in the past 12 months. Most of the training was provided when the employees were newly hired (n = 49; 44.5%) and only lasted 1-2 hours (n = 39; 35.4%). "Identifying food items that have allergens in the menus" (n = 66; 60.0%) and "Avoid cross-contact with food allergens" (n = 61; 55.5%) were topics most commonly included in employee training. Conversely, the respondents indicated "Communicating with back-of-the house about food allergies" (n = 49; 44.5%) was the least included topic in the training. The respondents identified "lack of commitment and interest among employees" as the biggest challenge that prevents them from initiating food allergy training. Policies to assist customers with food allergies included modifying recipes for customers with food allergies upon request (n = 85; 77.2%), stating food allergen on menus (n = 69; 62.7%), and posting food allergy information on restaurant websites (n = 52; 47.2%).

Significance: This study benefits the foodservice industries by identifying food allergy training needs and suggesting strategies to better serve customers with food allergies.

P3-153 A Breakthrough in Food Allergen Testing – Development of a One Minute Extraction Procedure Coupled to a Fast ELISA Assay

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Introduction: Persons allergic to certain food need to stick to an allergen-free diet and trust on product labeling for allergens. With allergens being the largest single cause of global product recalls, food manufacturers are seeking for fast and reliable methods ensuring the correct labeling of their products and preventing product recalls.

Purpose: Meeting these requirements, a new fast allergen ELISA assay was developed and validated for 8 different allergens, namely: Almond, Casein, Cashew, Egg, Hazelnut, Macadamia nut, Mustard and Peanut.

Methods: All eight allergen ELISA test kits incorporate an extremely fast extraction procedure – using only extraction capsules containing a proprietary powdered buffer and hot water – of only 1 minute and short incubation times in the ELISA assay of only 10 minutes. Besides a low cross reactivity, the kits also showed a good performance in intra and inter-assay precision validation with variation being below 15% in almost every kit.

Results: The limit of detection (LOD) was calculated based on the mean blank value of 19 blank extractions plus three-fold standard deviation. All kits have LODs ranging in the low mg/kg level (Almond: 0.5 mg/kg; Casein: 0.2 mg/kg; Cashew: 1 mg/kg; Egg: 0.5 mg/kg; Hazelnut: 1 mg/kg; Macadamia nut: 1 mg/kg; Mustard: 0.5 mg/kg; Peanut: 0.5 mg/kg). Recoveries were ranging from 64 – 130% when kits were challenged to recover spiked allergens in difficult food matrices such as chocolate, milk drinks, cookies and ice cream. The kits also showed an equivalent performance in recovery when being compared to established ELISA assays from the market.

Significance: The validation showed that these new developed allergen ELISA assays are not only capable providing results in an extreme fast way, but also yield accurate results that you can rely on, making them suitable tools for the detection of allergens in many different foodstuff.

P3-154 Differential Pharmacokinetics of Tetracycline and Sulfamethoxazole in *Ipomoea aquatica Forsk* Grown Hydroponically

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Introduction: Veterinary antibiotics in edible vegetables have quietly become an increasing food safety concern as these antibiotics may find their way back to the human food chain through vegetable consumption. Although much has been studied on the deposit levels of antibiotics in plants, relatively little is known regarding the fate of antibiotics with time once they got entry to the plant.

Purpose: To investigate the pharmacokinetics (uptake, distribution, metabolism and elimination) of two commonly detected environmental antibiotic residuals, tetracycline (TC) and sulfamethoxazole (SMX), in vegetable *Ipomoea aquatica Forsk*.

Methods: The vegetable was grown on 100 µg/ml of drug in cultivation fluid for 24 hours, drug concentrations in roots, stem and leaves at 0, 0.5, 3, 6, 12 and 24 hours were quantified by HPLC-UV method and the bioaccumulation factors (BAF) were calculated. Drug biotransformation was determined by LC/MS/MS. Subsequently, the vegetable was exposed to fresh cultivation fluids for evaluation of drug elimination.

Results: At 24 hours, for TC and SMX, the drug concentration was both highest in the root (30 and 6 µg/ml), followed by the leaves (10 and 5 µg/ml) and the stems (5 and 1 µg/ml). The BAF in tested tissues were either < 1 (TC) or < 0.1 (SMX), suggesting little accumulation. Drug biotransformation was not evident after one-week of growth. The elimination of drugs occurred mainly in the first 30 min, a total of 20% reduction for TC and 10% for SMX was recorded in 24 hours. Results were compared to those found in *Brassica Chinensis L.*

Significance: Differential pharmacokinetics existed for TC and SMX in *Ipomoea aquatic Forsk and was different in Brassica Chinensis L.* The ability of vegetables to quickly uptake micrograms of antibiotics in one day cannot be overlooked, but the deposition in the stems is minimal.

P3-155 An Evaluation of the Effectiveness of Water ATP Devices

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Introduction: Water ATP testing is important to several food and drink processes. It helps to monitor the quality of water used for food processing, evaluate the effectiveness of Clean-in-Place (CIP) rinses and control fermentation processes. It is therefore important that the water ATP device used is suitable for its intended purpose.

Purpose: To evaluate the performance of seven brands of ATP devices at different environmental temperatures and determine the ATP signal decay after one minute of activation.

Methods: The performance of the ATP devices was evaluated at temperatures ranging from 10°C to 35°C using an environmental chamber. All devices were tested using a solution which had a concentration of 5×10^{-9} M ATP. Testing was completed using the exact instructions provided by the device manufacturer. The signal decay was assessed by reading the result in the luminometers immediately after activation and at 60 seconds.

Results: 420 results were obtained to evaluate the temperature profile and ATP signal decay at one minute from activation. The results suggest that only two of the seven devices studied retained 80% of the maximum signal over a wide temperature range indicating that they were little affected by changes in ambient temperature. The results of the other five devices were highly temperature dependent. The results also indicate that for some devices, time within activation and reading the result in the luminometer has a considerable effect on the result.

Significance: The selection of the appropriate water ATP device for its intended environment temperature and the time taken to read the result in the luminometer following activation of the device is critical to ensure accuracy of the results.

P3-156 Stochastic Transfer of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during Preparation of Fresh-cut Salads in Household Set-up: Mathematical Modeling of Transfer

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Developing Scientist Competition

Introduction: Model-simulating bacterial transfer between household cutting-equipment and fresh produce is of high value for quantitative microbiological risk assessment of fresh-cut salads.

Purpose: To define the distribution of *Escherichia coli* O157:H7 and *Listeria monocytogenes* transfer rates between cutting-knives and lettuce leaves and model the bacterial transfer during consecutive cuts of leafy vegetables.

Poster

Methods: Lettuce leaves were inoculated with 5 log (HI) or 3 log (LI) CFU/g of *E. coli* O157:H7 or *L. monocytogenes* (serovars 1/2a, 4b). 100 sterile knives created single cuts to HI leaves (1 knife/cut). Subsequently, 100 knives, contaminated by cutting HI leaves, were used to cut uncontaminated lettuce leaves (UL), once/knife. Pathogens were enumerated on knives or lettuce and the % transfer-rate (TR%) from lettuce to knife or knife to lettuce, was determined. Next, a contaminated knife, performed consecutive cuts to UL and bacterial transfer from knife to lettuce was evaluated. A semi-mechanistic model, described the transfer of pathogens to knives and cut-vegetables during consecutive cuts. The model-performance was evaluated through sporadic introduction of inoculated lettuce leaves during the cutting process. Extrapolation experiments were also conducted (electric shredding of cabbage or knife-cutting of spinach and cabbage).

Results: Lettuce to knife TR-distributions were left-skewed, suggesting low transfer, for both pathogens. Knife to lettuce log TRs ranged from -2 to -0.4 for *E. coli* O157:H7 and -2 to 0 for *L. monocytogenes*, suggesting variable transfer potential. Regarding consecutive cuts, a rapid initial transfer was followed by an asymptotic tail at low cell numbers moving to lettuce or residing on knife. *E. coli* O157:H7 was transferred at slower rates than *L. monocytogenes*. These trends were sufficiently described by the transfer-model, with RMSE values of 0.421-0.675 and 0.337-0.466 for *E. coli* O157:H7 and *L. monocytogenes*, respectively. The model showed good performance in validation trials. However, during extrapolation experiments the model tended to underestimate bacterial transfer.

Significance: The present model could be a useful tool for quantitative risk assessment during preparation of leafy-green salads.

P3-157 Data Management Practices for Low and Negative Plate Counts Affect the Confidence Intervals of the Estimated Parameters of Microbial Reduction Models

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Developing Scientist Competition

Introduction: Experimental limits of detection (LOD) affect data available for developing microbial reduction models. To deal with this, published approaches include: (1) considering only positive values (Y_+), or replacing negative results by: (2) the LOD (Y_{LOD}), (3) one half the LOD ($Y_{LOD/2}$), (4) one divided by the LOD ($Y_{1/LOD}$), or (5) a random log number between 0 (1 CFU) and the LOD (Y_{RND}). Recent results show that these practices significantly affect accuracy of estimated parameters for log-linear and Weibull models.

Purpose: The objective was to quantify the effect of data management practices on confidence interval (CI) sizes for resulting model parameters.

Methods: Simulated experimental microbial reduction data sets (n = 100), with random errors, were synthesized (Y_{OBS}). The five low-count data management practices described above were applied. Log-linear and Weibull models were fit to the resulting data sets. The CIs of the parameters were estimated for each case, and then compared by ANOVA and Tukey.

Results: The ranking of CI sizes among data management practices varied among data and model types. The Y_+ approach, previously shown to be the most accurate (smallest RMSE, $P < 0.05$), nevertheless most often had the largest CIs, as much as double ($P < 0.05$) those for Y_{OBS}. For most of the other approaches, the CIs fell between those of Y_{OBS} and Y_+; 22 out of 30 cases yielded CIs greater ($P < 0.05$) than those for Y_{OBS}.

Significance: These results suggest that the application of low-count data management practices significantly affects both the accuracy and uncertainty of the model parameters. The fact that the CIs of Y_+ were most often the largest indicated that predictions based on these results, even if more accurate, are also more uncertain. This could influence model selection and utility in risk assessments and food safety management.

P3-158 Meta-analysis of Microbial Thermal Inactivation Response Data and Experimental Replication Errors via ComBase

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Introduction: Microbial inactivation models are built on the foundation of observed microbial responses to treatment variables over time. The impact of experimental and replication errors on model uncertainty is generally poorly reported; however, the resulting model accuracy and utility are inherently linked to the original experimental errors.

Purpose: The objective was to conduct a quantitative meta-analysis of experimental inactivation data reported in the Combined Database for Predictive Microbiology (ComBase), to examine factors affecting replication errors.

Methods: The entire collection of data in ComBase (current as of 18 March 2013) was acquired and consisted of ~45,000 data records, ~30,000 which contained time series data, and ~3,500 which were classified as thermal inactivation trials. A data mining and analysis program was coded (within Microsoft Excel) to identify and extract data from experiments reporting replicate data records, and then calculate replication error as a function of time, temperature, and source categorization. Data were discriminated by organism, product, product experimental conditions, methodology specifications, and temperature.

Results: Within the thermal inactivation records, beef (~19%) and laboratory media (~47%) comprised the largest fractions, and poultry, pork, produce, and low moisture foods (~5, ~2, ~2, and ~1%, respectively) a much smaller portion. Of the ~3,500 records, the median replication errors for experiments using poultry or pork were 0.045 and 0.028 log(CFU/g), respectively, while experiments using beef or produce were closer to the median global replication error across all inactivation records (0.184, 0.198 and 0.159 log(CFU/g)).

Significance: Based on the evaluation of ComBase records, there is a significant impact of test medium on replication errors in thermal inactivation studies. Additionally, there is a critical underrepresentation of key food categories in inactivation trials within the database.

P3-159 Quantitative Microbial Risk Assessment of *Clostridium perfringens* in Natural and Processed Cheeses

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Introduction: *Clostridium perfringens* spores generally exist in natural environment rather than vegetative cells. If *C. perfringens* spores exist in milk, the spores may not be destroyed by pasteurization, and thus, the spores may be still alive even in cheese.

Purpose: The purpose of this study was to evaluate the risk of *C. perfringens* foodborne illness for natural and processed cheeses.

Methods: To evaluate the risk of *C. perfringens* foodborne illness for natural and processed cheeses, the hazard of *C. perfringens* was identified through literatures, and dose response models were also searched to characterize the hazard of the pathogen. For exposure assessment, prevalence of the pathogen, storage temperatures, storage time, and annual amounts consumption were surveyed. Subsequently, a simulation model was developed using the collected data, and the simulation result was used to estimate the probability of illness with @RISK.

Results: *C. perfringens* was determined to be low risk group on cheese by hazard identification, and the exponential model ($r=1.82\times10^{-11}$) was appropriate for hazard characterization. Annual consumption amounts of natural and processed cheeses were 11.69±11.22 g and 19.43±15.38 g, respectively. The contamination level of *C. perfringens* on cheeses was below detection limit (< 0.30-0.45 log CFU/g), and initial contamination level was estimated by beta distribution (α_1=1, α_2=91) to be -2.35 log CFU/g. Moreover, there was no growth of *C. perfringens* spores during distribution and storage. These data were used for risk characterization by a simulation model, and the mean and maximum values of the probability of *C. perfringens* foodborne illness per person a day on natural and processed cheeses were 9.57×10^{-14} and 3.58×10^{-14}, respectively.

Significance: These results can be used to establish a microbial regulation for *C. perfringens* on natural and processed cheeses.

P3-160 Microbial Risk Assessment of *Escherichia coli* in Cheese Supply Chain

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Introduction: *Escherichia coli* strains have been isolated from various cheeses, and thus, several studies for the microbial risk assessment have been conducted. However, most microbial risk assessment for *E. coli* in cheeses did not include entire food chain.

Purpose: The objective of this study was to evaluate the microbial risk of foodborne illness of *Escherichia coli* be consuming various cheeses in entire food chain.

Methods: To identify the hazards of *E. coli*, literatures and foodborne outbreaks linked to *E. coli* were reviewed. In hazard characterization, the probabilities of distribution for storage temperature and time during cheese distribution and dose response models for *E. coli* were searched. The consumption pattern for cheese was also investigated. For exposure assessment, the contamination level of *E. coli* on cheeses was evaluated along with a cheese supply chain, and maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h) were calculated with predictive models developed in other studies. Subsequently, simulation models were developed, and the probabilities of illness per person a day were calculated.

Results: Hazard of *E. coli* in cheese was identified, and consumption cheese pattern was collected, and the prevalence of *E. coli* was determined to be very low along with cheese supply chain. Predictive models estimated that the μ_{max} and *LPD* of natural cheese were 0.0-0.94 log CFU/g/h and 5.40-1320.0 h, and μ_{max} and *LPD* of processed cheese were 0.0-0.33 log CFU/g/h and 1.79-1320.0 h, respectively. A simulation model developed with the results of hazard identification, exposure assessment, and hazard characterization estimated the mean probabilities of illness per person a day to be 1.36úl10^{-7} and 2.12úl10^{-10} for natural and processed cheeses, respectively.

Significance: These results should be useful in providing the scientific data for establishing microbial criteria for *E. coli* in cheeses.

P3-161 Use of a Quantitative Risk Assessment Model to Estimate Exposure to *Salmonella* Associated with Dry Pet Foods

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Introduction: Recent *Salmonella* outbreaks associated with dry pet foods and treats highlight the importance of these foods as previously overlooked exposure vehicles for both pets and humans. While the industry has upgraded equipment, cleaning protocols, and product testing, no comprehensive assessment of the risk profile of these products or the effectiveness of current *Salmonella* control measures is available.

Purpose: This study sought to develop an ingredients-to-consumer quantitative microbial risk assessment model to: 1) estimate pet and human exposure to *Salmonella* via dry pet food, and 2) assess the impact of industry and household-level mitigation strategies on such exposure.

Methods: Data on prevalence and concentration of *Salmonella* in pet food ingredients, production process parameters, bacterial ecology, and contact transfer in the household were obtained through a literature review, industry data, and experiments. An agent-based probabilistic Monte Carlo modeling framework was built in the @Risk Excel™ add-in.

Results: Human exposure due to handling pet food is minimal if contamination occurs before extrusion (mean dose 6×10^{-16} CFU/exposure event, with initial 2,500 CFU/kg in protein meal ingredient). Exposure increases considerably if such contamination levels occur in coating fat bypassing extrusion (mean dose 6×10^{-4} CFU/event). The outcome is highly variable, spanning 9 log CFU. Recontamination after extrusion and coating, e.g., via dust or condensate, can also lead to high exposure (mean dose 4×10^{-2} CFU/event with fixed 2,500 CFU/kg contamination in intermediate product). Hand washing after handling food can reduce dose by about 1 log. Exposure is the highest when *Salmonella* is transferred to human wet food, left at room temperature (dose 8×10^{-2} CFU after 2 hours, when coating fat is contaminated).

Significance: This model allows quantifying exposure associated with dry pet food, under current and alternative production and handling practices. Results can inform improved industry control measures, risk communication to consumers, and regulations.

P3-162 Effect of Storage Temperatures on the Survival of *Salmonella* and *Campylobacter* in Chicken Eggs

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Introduction: Human infections with *Salmonella* and *Campylobacter* are mainly from consumption of raw or undercooked eggs and poultry. Foodborne illness caused by *Salmonella* is a worldwide problem. *Salmonella* contaminated eggs present a hazard for consumers during handling; contaminated eggs can contaminate other surfaces during food preparation. *Campylobacter* strains were used in this study, even though there is disagreement among scientists regarding egg passage of *Campylobacter* to poultry through eggs.

Purpose: The objective of this study was to evaluate the survival of inoculated *Salmonella* and *Campylobacter* on egg shells and egg contents.

Methods: The initial *Salmonella* and *Campylobacter* egg inoculation levels were 2 log CFU/egg and 4 log CFU/egg for both external and internal contamination. *Salmonella* and *Campylobacter* inoculated eggs were subjected to different storage temperatures: 30°C and 23°C for 0, 6, 24, 48 hours and 4°C for 1, 7, 14, and 21 days. Confirmation of *Salmonella* and *Campylobacter* isolated from eggs were subjected to Polymerase Chain Reaction.

Results: Generally, there was a decline on the levels of *Salmonella* on shell eggs. Recovery of *Salmonella* at 4°C for 1, 7, 14, and 21 days from internal contamination was between 4.76 -2.56 CFU/ml. *Salmonella* population increased (6.00– 7.51 CFU/ml) in internal contaminated eggs at 30°C and 23°C. No viable *Campylobacter* cells were indicted on shell eggs stored at 4°C, even after the enrichment. For internal contamination, *Campylobacter* populations decreased (3.24 – 2.32 CFU/ml) for eggs stored at 4°C for 6 hr and 1 day. At day 14, the bacterium was not detected even after enrichment.

Significance: The present study demonstrates *Salmonella* in eggs can multiply at room temperature and unrefrigerated conditions. *Campylobacter* can survive inside the egg contents for up to 14 days after internal contamination. Results of this study reinforce the critical importance of egg refrigeration in order to protect consumers from *Salmonella* and *Campylobacter* infections.

P3-163 Evaluation of Quantitative Microbial Risk Assessments for *Salmonella* and *Campylobacter* in Poultry Meat

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Introduction: Poultry meat, which constitutes a large portion of American diet, is of great food safety concern as poultry has been recognized as a major vehicle for foodborne pathogens such as *Salmonella* and *Campylobacter*. In recent years, several quantitative microbial risk assessment models for poultry meat have been developed to control and manage the risk of *Salmonella* and *Campylobacter*.

Purpose: The objectives of this study were to provide a review of quantitative microbial risk assessment models for poultry developed in recent years to summarize available data and information, and to identify data gaps that are needed for future research.

Methods: A broad literature search was done, and quantitative microbial risk assessment models for both *Salmonella* and *Campylobacter* in poultry meat and poultry meat products developed in the United States, Belgium, Canada, United Kingdom, Denmark, the Netherlands and Sweden were compared and discussed.

Results: All models include some or all main stages of the poultry meat production chain: primary production, industrial processing, preparation, and consumption. The modelling approaches vary between these risk assessment studies, including the use of different growth and dose-response models. Cross contamination and undercooking are major concerns for the risk of pathogen contamination during consumer preparation stage. Intervention strategies aimed at reducing pathogen concentration at retail level, reducing the incidence of undercooking, and appropriate handling of poultry meat and their products are effective in controlling the risk associated with *Campylobacter* and *Salmonella* in poultry meat.

Significance: This review summarized existing models and available data for quantitative risk assessment studies of *Campylobacter* and *Salmonella* in poultry meat. Critical data gaps such as pathogen behavior at primary production stage, and time/temperature data between processing and retail were identified. This study could serve as a useful resource for future quantitative risk assessment studies of pathogens in poultry meat.

P3-164 Qualitative Risk Assessment of *Toxoplasma gondii* Infection from Meat Consumption in the United States

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Introduction: Toxoplasmosis, caused by *Toxoplasma gondii*, was identified as the fourth leading cause of hospitalizations and the second leading cause of deaths among 31 major foodborne pathogens in the U.S. with estimated 4,428 hospitalizations and 327 deaths annually. It was estimated that approximately 50% of *T. gondii* infections were from ingestion of meat products.

Purpose: The objectives of this study were to develop (i) a risk profile that provides the background information of *T. gondii* infection, and (ii) a risk assessment framework that qualitatively analyze the risk caused by *T. gondii* from varied meat products consumed in the U.S.

Methods: Relevant studies were collected by searching PubMed and Google Scholar. Meta-analysis was used to calculate average weighted prevalence. Risk estimation of different meats was analyzed by a qualitative risk assessment based on a farm-to-retail framework.

Results: Based on the weighted prevalence, it was found that risk for meats from free-range chicken, goat and lamb is higher than that from pig, cattle, and caged-chicken. For fresh meat products, risk remained high at retail level unless meats had been frozen or moisture-enhanced. Our results showed that meat processing such as salting, freezing, and cooking were able to inactivate *T. gondii* cysts in meat products, while nitrite/nitrate, spice, drying, fermentation, low pH, and cold storage had no effect on the viability of *T. gondii* cysts. Thus, raw-fermented sausage, cured raw meat, dried meat products and fresh processed meat products are associated with higher infection risks compared with cooked meat products.

Significance: Risk profiling provided comprehensive knowledge of *T. gondii* infection. Specific meats linked to human toxoplasmosis were identified through the qualitative risk assessment. This study would serve as a reference for meat management control programs to determine the critical control points, and the foundation for future quantitative risk assessments.

P3-165 Apple Growing and Packing Risk Factors and Their Potential to Lead to Foodborne Illness Outbreaks

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Introduction: Although apples have a reputation with consumers as being a safe commodity, the current need in the fresh market apple industry is for growers and packers to better understand their risk potential in order to increase the effectiveness of their mitigation practices and ensure the continued delivery of safe fresh market apples to consumers.

Purpose: The purpose of this study was to evaluate microbial food safety risks and potential health effects associated with the application of evaporative cooling water to orchards using quantitative microbial risk assessment (QMRA).

Methods: Data from scientific studies and industry were used to identify potential pathogens of concern and *E. coli* O157:H7 was selected due to data availability. A QMRA model was developed using Microsoft Excel and Palisade @Risk to estimate the probability of illness due to exposure from fresh market apples. Several scenarios were evaluated to assess the impact of concentration and prevalence in cooling water, time between cooling and harvest, and use of detergents, sanitizers, and wax use on final concentration and prevalence rates, and a sensitivity analysis was conducted to identify crucial mitigation steps.

Results: The probability of illness at estimated prevalence rates of contamination was determined to be 1.5 cases in 100 million servings in the general adult population, based on a worst case scenario. Production practices were shown to be important contributors to risk levels.

Significance: QMRA is a useful tool in estimating human health impact and for affirming the importance of critical control steps in fresh market apple production. Understanding how the production and packing processes affect pathogen levels, to the extent pathogens are present, provides the industry with a means to evaluate the practices they currently employ, and an opportunity for adjustments if QMRA results indicate that current practices are insufficient at reducing the potential for illness.

P3-166 Investigation of Hazards from Kiwi Fruit and Their Cultivation Areas to Establish a Good Agricultural Practices (GAP) Model

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Introduction: Many outbreaks of foodborne illnesses have been associated with the consumption of fresh vegetables and fruits contaminated with foodborne pathogens. Kiwi fruit is potential to be contaminated with bacteria.

Purpose: This study was to validate microbiological hazards of kiwi fruit and their cultivation areas and to suggest recommendations for the development of a good agricultural practices (GAP) model.

Methods: A total of 72 samples were collected from cultivation environments (soil, agricultural water, and atmosphere), plants (kiwi fruit and its leaf), personnel hygiene (glove, cloth, and hand), agricultural products processing center (tray loader, conveyer belt, weighing cup, collector, box) located in Gyeongnam, Korea, and were tested to analyze sanitary indicator bacteria (aerobic plate counts, coliforms and *Escherichia coli*), major foodborne pathogens (*E. coli* O157:H7*, Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus,* and *Bacillus cereus*), and fungi.

Results: Total bacteria, coliform, and fungi in the kiwi fruit farms were detected at the level of 0.8~6.7, 0.5~4.6, and 0.3~4.7 log CFU/g (or leaf, ml, hand, 100cm^2), respectively. In case of pathogenic bacteria, *B. cereus* and *S. aureus* were detected at levels of 1.0 ~ 4.9, and 1.0~1.7 log CFU/g (or leaf, ml, hand, and 100 cm^2) in samples, but other pathogen bacteria including *E. coli* O157, *L. monocytogenes*, and *Salmonella* spp. were not detected in any samples.

Significance: According to the results, the kiwi fruit sampled from farms and agricultural products processing center were comparatively safe with respect to microbial hazards. However, cross-contamination of bacteria from environments and workers could be potential risks which give harm to consumer. Therefore, to minimize microbial contamination and to ensure the safety of kiwi fruit, it is necessary proper GAP model should be established.

P3-167 Pecan Truffles (*Tuber lyonii*), an Edible North American Truffle Species: Flavor Characterization, Impact of Storage Condition on Volatile Organic Compounds, and Heavy Metal and Pesticide Concentration

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Developing Scientist Competition

Introduction: *Tuber lyonii* is a North American truffle species commonly known as "Pecan Truffle." It is a choice edible fungi species with pleasant aroma that has not been well characterized sensorially or chemically.

Purpose: This study was proposed to (1) characterize *T. lyonii* aroma (2) evaluate the influence of storage conditions on the concentrations of volatile organic compounds (VOCs) in truffle fruits, and (3) measure heavy metal and pesticide contents.

Methods: Ten trained judges evaluated eight aroma sensory attributes (banana, beet, black olive, parmesan cheese, garlic, mushroom, nail polish remover, and plastic) in *T. lyonii*, black Périgord truffle (*T. melanosporum)*, and Italian white truffle (*T. magnatum*). Data were analyzed using analysis of variance (ANOVA). The effect of storage time (1 to 7 days) after harvest and three storage methods (paper bag at 4°C, glass jar at 4°C, and glass jar at -18°C) on VOC content in truffles was quantified using a headspace stir bar sorptive extraction gas chromatography mass spectrometry method (HS-SBSE-GC-MS). Heavy metal content (As, Cr, Hg, Cd, Pb) was measured by inductively-coupled plasma mass spectrometry. Samples were screened for more than 150 pesticides using the California Department of Food and Agriculture (CDFA) multi-residue method.

Results: *T. lyonii* was characterized as being similar to *T. melanosporum* but with less nail polish aroma. VOC content in *T. lyonii* may decrease significantly after harvest, depending on the storage method. Storage in air-tight glass jars at -18°C was most effective at preserving VOCs. Cr (0.21-0.36 mg/kg) and Cd (0.39-1.10 mg/kg) were detected in *T. lyonii*, while As, Hg, and Pb were below the limits of detection. No pesticides were detected in aggregate truffle samples from multiple locations.

Significance: This is the first aroma characterization of pecan truffles. These results did not indicate risk from heavy metals or pesticide residues.

Poster

P3-168 Microbiological Hazard Analysis of Sweet Persimmon Farms to Develop a Good Agricultural Practices (GAP) Model

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Introduction: The incidence rate for illnesses associated with the consumption of fresh fruits has been continuously increased. Sweet persimmon could be one of the fresh fruits to develop outbreaks of foodborne illnesses.

Purpose: Objectives of this study were to assess microbial risks of sweet persimmon farm and their cultivation areas and to provide basic data for the establishment of a good agricultural practices (GAP) model.

Methods: A total of 74 samples were collected from cultivation environments (soil, irrigation water, and atmosphere), plants (sweet persimmon and its leaf), personnel hygiene (glove, cloth, and hand), agricultural products processing center (tray loader, conveyer belt, weighing cup, collector, box) located in Gyeongnam, Korea, and were tested to analyze sanitary indicator bacteria (aerobic plate count, coliforms and *Escherichia coli*), major foodborne pathogens (*E. coli* O157:H7*, Listeria monocytogenes, Salmonella* spp.*, Staphylococcus aureus,* and *Bacillus cereus*), and fungi.

Results: Total bacteria, coliform, and fungi in the sweet persimmon farms were detected at the level of 0.5~6.9, 0.5~5.6, and 1.0~5.1 log CFU/g (or leaf, ml, hand, 100cm^2), respectively. Only soil was confirmed to be *E. coli* positive. In case of pathogenic bacteria, *B. cereus* was detected at levels of 1.0 ~ 4.4 log CFU/g (or ml, hand, and 100 cm^2) in some samples. *S. aureus* was maximum detected level of 2.0 log CFU/100cm^2 in carrier box. But other pathogen bacteria including (*E. coli O157*, *L. monocytogenes*, and *Salmonella* spp.) were not detected in any samples. Although *E. coli* were detected in irrigation water, the level of microbial was lower than the regulation limit.

Significance: According to the results, the microbial population on the sweet persimmon was detected at lower level as compared with the standards set by Korean government. However, cross-contamination of microorganism by cultivation environments and worker has been generally reported, an efficient GAP model is one of the most important things to improve the safety of sweet persimmon for microbiological hazards. Therefore, the results could be used as basic data to establish GAP model for providing safer sweet persimmon than before.

P3-169 Modeling Growth of *Listeria monocytogenes* in Sanitizer-treated Diced Onions, Tomatoes and Celery

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Introduction: Predictive modeling of microbial growth is an important tool in risk analysis with product-specific growth parameters needed for reliable predictions.

Purpose: Given recent safety concerns with fresh-cut produce, this study aimed to model *Listeria monocytogenes* growth in sanitizer-treated diced onions, tomatoes and celery during storage at 4, 7 and 12°C.

Methods: Retail 8-kg batches of onions, tomatoes, and celery were washed, diced (Nemco Slicer Model 55500-2, 3/8 inch blade), and then dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F, and J29H) to obtain ~4 log CFU/g. After 8 min of draining, the samples were immersed in 80 ppm chlorine (XY-12, Ecolab) adjusted to pH ~6.0 or tap water for 2 min, centrifugally dried, and aerobically packaged. *L. monocytogenes* was periodically enumerated on Modified Oxford Agar during 18 d of storage at 4, 7,12, 16, or 23°C with the Baranyi and Robert model used to describe growth.

Results: As expected, none of the samples exhibited significant growth at 4°C. At 7°C, *Listeria* populations increased 1 - 3 logs after 18 d of storage with diced water-treated onions showing the highest populations. However, these growth data were insufficient for modeling. The Baranyi and Robert model predicted a ~5-log increase for all samples stored at 12, 16, or 23°C, with the maximum growth rates ranging from 0.0251 log/day for diced onions at 16°C to 0.33 log/day for samples stored at 12°C. The laboratory growth and model predictions were similar for all samples with the root mean squared error (RMSE) and bias respectively ranging from 0.2762 for onions at 23°C to 0.6321 for celery stored at 16°C, and from -0.00947 for tomatoes at 12 °C to 0.03105 for onions at 23°C.

Significance: These findings will be useful to risk assessors in predicting human exposure to *Listeria* from contaminated onions, tomatoes and celery.

P3-170 Modeling the Effect of Temperature and pH on the Growth of *Salmonella* in Cut Tomatoes

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Developing Scientist Competition

Introduction: Outbreaks of salmonellosis associated with fresh cut produce have been an increasing food safety concern recently and tomato is one of the popular produce. When tomatoes are cut, *Salmonella* can be transferred from the tomato surface to the flesh, where growth can occur.

Purpose: The manipulation of pH and storage temperature provides one feasible method for Salmonella control in cut tomato products. The purpose of this research was to expand our existing understand of *Salmonella* growth in fresh cut tomatoes, resulting in regression models able to predict both growth rate and lag time of *Salmonella* as a function of pH and temperature.

Methods: Whole red round tomatoes were dip-inoculated in a mixture of four Salmonella strains obtained from the CDC, which 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 at 5-degree intervals. The pH of the cut tomatoes was adjusted from 3.7 to 4.4 by the addition of 5% citric acid. Samples were enumerated by plate counts on XLT4 agar until Salmonella growth reached stationary phase. Growth rates and lag time were calculated by an Excel add-on DMFit. Regression models were built by SAS.

Results: Both the growth rate and lag time of Salmonella were described as multivariate regression functions of temperature and pH with good fit (both with $P < 0.0001$ & $R^2 > 0.40$). The probability of the end of lag time was described as a logistic function of time, temperature and pH with a good fit ($P < 0.0001$).

Significance: The models of growth rate and lag time of Salmonella in cut tomatoes built in this project provide useful tools in the risk assessment and risk management of estimating the risk by different temperature abuse and pH manipulation.

Poster

P3-171 *Salmonella* Serotypes in Central Florida Surface Waters

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Introduction: Identification of environmental *Salmonella* serotypes is essential to understand sources and potential diversity of in-field contamination. *Salmonella* in surface water can present a food safety risk through indirect consumption of exposed horticultural crops.

Purpose: This work identifies *Salmonella* serotypes associated with Central Florida surface waters over a twelve-month period.

Methods: Water samples (10 l) were taken monthly, for 12 months, from 18 locations (202 samples). *Salmonella* was enumerated by MPN using a modified FDA-BAM *Salmonella* method using 33.3 ml of the water sample. Up to 36 isolates from each water sample were determined to be either different, or likely the same, using multiplex PCR patterns and serogrouping. All *Salmonella* isolates from each water sample determined to be different using multiplex PCR patterns and serogrouping, were serotyped and analyzed with PFGE.

Results: *Salmonella* isolates (223) were cultured from 81.7 % (165/202) of water samples. Of the serotypes identified (32), the most frequently isolated included Muenchen (11.5%), Rubislaw (9.5%), Anatum (8.8%), Gaminara (8.8%), and IV_50:z4,z23:- (6.8%). Each *Salmonella*-positive water sample yielded at least two, and at most 11, different serotypes; the average number of serotypes from a *Salmonella*-positive water sample was six. Serotypes most frequently isolated during each monthly sampling period included Anatum (8/12), Muenchen (7/12), and Rubislaw (6/12). No single serotype was isolated during every monthly sampling period from any of the sampling locations and no temporal patterns were observed. The majority (143) of isolates, including some from all sampling locations, clustered into one genotypic group (>80% similar) by PFGE analysis; this group included 23 of the 32 serotypes.

Significance: *Salmonella* in Central Florida surface waters are serotypically diverse and include clinically common serotypes. The use of untreated surface water for agricultural purposes that contacts the harvested portion of edible horticultural crops close to time of harvest should be considered a food safety risk.

P3-172 Gluten Contamination in Foodstuff Labeled as "Gluten-free" in the United States: A Pilot Study

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Introduction: Gluten is the main storage protein in grains and consists of gliadin and glutelin occurring in the same ratio. Persons suffering from intolerances including celiac disease (CD) must avoid foods containing gluten or products containing wheat, barley, and rye. Accordingly, gluten detection is of high interest for food safety of celiac patients.

Purpose: This study was designed to determine concentrations of gluten in foods labeled as 'gluten-free' available in United States.

Methods: Seventy-eight samples labeled as 'gluten-free' were collected and analyzed using a gliadin competitive enzyme-linked immunosorbent assay (ELISA). The calculation of gluten is based on the assumption of the same ratio between gliadin and glutenin.

Results: Forty-eight of the 78 (61.5%) samples contained less than the limit of quantification (LOQ) of 10 mg/kg for gluten. Fourteen of the 78 (17.9%) labeled as 'gluten-free' samples contained less gluten than the guidelines established by the Codex guide line for gluten-free labeling (20 mg/kg). Sixteen samples (20.5%) did contain gluten levels ≥ 20 mg/kg, with gluten levels ranging from 20.3 mg/kg to 60.3 mg/kg; in particular, 5 out of 8 breakfast cereal samples showed gluten contents higher than 20 mg/kg.

Significance: These results are alarming as gluten sensitivity is known to vary among CD patients.

P3-173 Survival of Vegetative Pathogens in Model Flavor Systems Containing Medium Chain Triglycerides

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Introduction: The most commonly used carrier compounds in flavor-based systems in the food industry are ethyl alcohol, propylene glycol, medium chain triglycerides, or triacetin. Previously we have presented a study on the antimicrobial properties of ethyl alcohol, propylene glycol, and triacetin in food flavor systems. The new study was undertaken to evaluate the bactericidal effect of medium chain triglycerides at different concentrations on the survival of various vegetative pathogens.

Purpose: The objectives of this study were: (1) to evaluate the antimicrobial properties of medium chain triglycerides on the survival of *Salmonella* spp., *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*, (2) to find the optimum concentration of this carrier compound that may be bactericidal (ex: 5-log reduction) for vegetative pathogens

Methods: Cultures (1 g) of *S.* Typhimurium, *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes* were added to 9 ml of glycerol with the medium chain triglyceride concentrations of 50, 60, 70, 80, and 90% to achieve an initial inoculation of 6-7 log CFU/ml. All the sample concentrations were performed in duplicate. 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 90% for medium chain triglycerides.

Significance: The current study provides additional scientific basis for the antimicrobial effect of different chemical carrier compounds routinely used in food flavor systems.

P3-174 Study on Risk Evaluation Model for Food Facilities Based on Analytical Hierarchy Process

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Introduction: According to statistics, the number of permitted food manufacture in China maintains at about 120 thousands. There is a wide difference in processing-level between food enterprises, with some modern food processing enterprises reaching international standards and some small workshops depending on traditional experience in processed foods. Also, there is a difference in management and processing techniques between enterprises. So these differences place higher demands on the supervision in China. The method to identify the most important emphases places a big challenge in supervision.

Purpose: This paper initiatively proposes the establishment of food production enterprises risk index system, then utilizes the analytic hierarchy process (AHP) to calculate risk relative weight, constructs the establishment risk classification of the safety supervision model, finally applies this model in investigating, sorting, classifying, and calculating the production enterprise's data to achieve the quantification the enterprise safety supervision cycle.

Methods: analytic hierarchy process (AHP)

Results: A five-key-factors evaluation method was studied for risk ranking of food categories, and the weight of each factor was evaluation by 30 scientists. The risk ranking of food categories was given based on five-key-factors evaluation method. Second, the most important subject behavior of food enterprises were identified based on risk surveillance results from 2009 to 2012, and a risk-based inspection method was established for supervisors.

Significance: This research gives an efficient method for China Food and Drug Administration (CFDA) to establish a new supervision system on food enterprises.

P3-175 A Preliminary Risk Assessment of *Bacillus cereus* Isolated from Taiwan Foodborne Illness Outbreaks and Food Survey

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Introduction: The top three causes of foodborne illness outbreaks (Taiwan 2002-2012) were *Vibrio parahaemolyticus, Staphylococcus aureus* and *Bacillus cereus*; the reported outbreaks/illnesses were 647/9228, 232/5872, and 176/5691, respectively. In 2010-2012, outbreaks associated with *Bacillus cereus* increased significantly, accounting for 26.0%/47.4% of all outbreaks/illnesses (404/7958) caused by bacteria pathogens. The risk factors contributing to this increase remain to be determined.

Purpose: *Bacillus cereus* food poisoning is typically associated with toxins. The diversity of *Bacillus cereus* strains with different toxins is reflected in their broad range of pathogenicity. We conducted a preliminary risk assessment of the *Bacillus cereus* isolated from Taiwan foodborne illness outbreaks and food survey by characterizing their toxin-producing genes and toxin profiles.

Methods: *Bacillus cereus* were isolated from outbreak-associated food samples and food survey samples by culture methods and identified with biochemical reactions. The toxin-producing genes for diarrheal enterotoxins (hemolysin BL, non-hemolytic enterotoxin, enterotoxin FM, enterotoxin T, and cell toxin K), and emetic toxin (cereulide) were detected by PCR methods coupled with specific primers for each toxin gene. Immunoassay test kits were used to determine diarrheal toxins, including non-hemolytic enterotoxin and hemolysin BL. Since there was no commercial kit for emetic toxin, a LC-MS method was implemented.

Results: The frequency of *Bacillus cereus* detected from outbreak-associated samples was around 9%. Among the 189 isolates examined, almost all of them contain one or more toxin-producing genes, but we could not find any isolate that produced emetic toxins. The prevalence of *Bacillus cereus* in food survey samples was around 16%, and most (98%) were contaminated at a level < 100 CFU/g. However, there were 3 (1.6%) isolates that produced emetic-toxin among the 123 isolates examined.

Significance: Toxin-producing strains of *Bacillus cereus* show distinct characteristics between different strains. Total counts of *Bacillus cereus* in food survey samples were low, but the emetic-toxin-producing strains could pose potential risk.

P3-176 *Escherichia coli* O157 in Finnish Slaughter Cattle

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Introduction: The Finnish EHEC Control Programme (FECP) for cattle was launched in Finland in 2004. According to it, faecal samples of individual, randomly chosen animals are to be collected at slaughterhouses evenly year-round in a way that reveals a 1% prevalence of *E. coli* O157 with an accuracy of 0.5% and a confidence level of 95%. Positive animals must be traced back to the farms and restrictive measures activated.

During the years, the apparent prevalence of slaughter animals has varied from 0.20% (2008) to 2.1% (2013) with sample sizes 1495 and 1570, respectively. An evaluation of the effectiveness of the FECP was conducted in 2010 based on 2006-2010 data, revealing both incapability of the system to detect infected animals or herds and unsteadiness in its implementation.

Purpose: After several years with a relatively low O157 prevalence, the results of the years' 2012-2013 FECP showed about 3-fold increase. A new study was therefore started to find out whether the outcome resulted from actual increase of the prevalence or weaknesses of the programme.

Methods: True prevalences on annual data sets of the FECP were assessed with WinBUGS 1.4.3. Test sensitivity 0.988 was included.

Results: The true prevalence among slaughter animals was lowest in 2008 (95% CI: 0.074%-0.58%) and highest in 2013 (95% CI: 1.53%-2.99%). The number of O157 infections among slaughter cattle in 2012 and 2013 showed a significant increase compared to the previous years. However, the O157 level of low-prevalence years differed significantly only from the high-prevalence years 2012-2013.

Significance: According to the statistical analysis, the increase of O157 within the FECP during 2012-2013 was true. Because previous evaluation of the FECP already revealed many deficiencies, e.g., in detection probability and sampling implementation, the conclusions about the trend cannot be based on annual FECP results without detailed examination of the data.

P3-177 Intake of Estragole and Trans-anethole from Fennel-containing Plant Food Supplements among Finnish Consumers

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Introduction: Plant food supplements (PFS) have a long tradition of use, and they are widely consumed in many European countries. However, little is known about the risks and benefits associated with the consumption of PFS. Fennel (*Foeniculum vulgare)* is a perennial aromatic herb, which has been used in traditional medicine to treat many kinds of symptoms. According to the results of the PFS Consumer Survey conducted in Finland, Germany, Italy, Romania, Spain and the United Kingdom, fennel ranked sixth in the pooled list of most consumed botanicals. The essential oil of fennel contains trans-anethole and estragole, among other compounds. Both have exhibited antimicrobial activity, but trans-anethole also has tumorigenic properties in laboratory animals, and estragole is carcinogenic and mutagenic at high doses.

Purpose: The objectives of the present study are to describe the consumption of PFS that contain fennel among a sample of Finnish consumers of PFS and to estimate the intake of estragole and trans-anethole from PFS with fennel among the Finnish consumers.

Methods: A cross-sectional 12-month retrospective survey was conducted in Finland and five other European countries. The sample size in Finland was 401 PFS consumers. They were from four cities located in different parts of the country. The study participants were regular PFS consumers.

Results: The estimated average intake of estragole was 0.20 mg/d (range 0.01-7.95 mg/d), and of trans-anethole 1.15 mg/d (range 0.05-63.1 mg/d).

Significance: The intake of estragole from PFS with fennel was moderate when compared with intake from other dietary sources. The intake of trans-anethole did not exceed the ADI value in the present study even in the upper range of intake. However, the individual variation of intake was quite large.

P3-178 Microbial and Heavy Metal Contamination in Street Food Vending Business in Uganda

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Introduction: Street-food vending is becoming important part of urban and peri-urban lifestyle in Uganda. However, street foods have been implicated in several outbreaks of foodborne diseases.

Purpose: Microbial and heavy metal contamination associated with street food business in Kampala, Jinja and Masaka districts was assessed.

Methods: Standard microbiological methods were used to determine coliforms, *S. aureus*, *E. coli*, *B. cereus*, *Campylobacter* spp., *Pseudomonas* spp., and *Salmonella spp.*counts. Selected street foods, use-water, food vendor's hands, utensils, final dish rinse-water and serving surfaces were tested. Heavy metal (Cadmium, Lead, Zinc, Copper and Nickel) levels were determined using Atomic Absorption Spectrophotometer. Swabbing method was used to determine surface contaminations. A total of 60 samples were tested

Results: *S. aureus,* coliforms and *E. coli* ranged between <1 and 6.18 ± 0.24 log CFU/g (cm^2, ml). *S. aureus* counts were significantly different ($P < 0.05$) amongst street foods. *S. aureus,* coliforms, *E. coli*, *B. cereus, Campylobacter* spp., and *Pseudomonas* spp in use-water were <1 log CFU/ml. Coliforms and *S. aureus* counts on serving surfaces, vendors' hands and serving utensils, use-water and final dish rinse-water ranged between < 1 to 4.22 ± 1.81 log CFU/cm^2 (ml). No significant difference ($P > 0.05$) in *E. coli* counts detected on serving surfaces and utensils. *Bacillus cereus*, *Campylobacter spp.* and *Pseudomonas spp.* were <1 log CFU/g (ml). Heavy metal levels varied from 0.01 to 9.4 ppm. In all the districts, bean and groundnut sauce had the highest cadmium concentrations. Bean sauce and chapatti samples had significantly different ($P < 0.05$) Copper levels among the districts. Nickel was undetectable. Use-water collected from Kampala district had highest Cadmium (0.36 ppm), Zinc (1.68 ppm) Lead (7.6ppm) and Copper (2.08ppm).

Significance: Some of the street foods and surfaces constituted a health risk due high microbial numbers and heavy metal levels. There is need for conducting a risk assessment and sensitization of vendors on basic food safety practices and behavioral change.

P3-179 A Rapid Method for Sampling and Enumeration of Airborne Mold Spores

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Introduction: It is important to monitor air quality in food processing environment in order to control microbial contamination and ensure food safety. Level and composition of airborne fungal spores is a good indicator of microbial indoor air quality. This presentation further evaluates the potential of using elevated spore levels in indoor air to assess undesirable damp conditions that may foster microbial growth.

Purpose: This study analyzes airborne fungal spore data collected from 1,200 indoor environments to examine correlations between airborne spore concentration and surface mold growth in the same environments.

Methods: Sampling of airborne fungal spores was performed using a slit impaction method. Fungal spores in the air stream were collected onto glass slides. In the laboratory, the glass slide containing the sampling media were examined with optical light microscopy. Fungal spores were counted and identified and results were expressed as spores per cubic meter of air. Surface microbial samples were taken from the same indoor environment and categorized into various groups according to the levels of contamination. Using this method, 1,200 air and surface samples were taken and analyzed, and relationships between the two data sets were assessed.

Results: This study showed that there was a close correlation between onsite of surface mold growth and elevated airborne spore level. The data indicated that the detection of significant levels of airborne *Aspergillus, Penicillium, Chaetomium*, and *Stachybotrys* spores were a good indication of surface mold growth in the environment.

Significance: Surface microbial growth is an undesirable condition for food manufacturers. However, some surface microbial growth may be hidden or behind walls, making in time detection difficult. This study shows that sampling of airborne fungal spores may be helpful to locate hidden mold growth and assess microbial air quality.

P3-180 Tracking and Modeling of *Listeria monocytogenes* Contamination in Spinach Fields from Planting to Harvest

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Developing Scientist Competition

Introduction: Meteorological factors have been shown to influence the risk of foodborne pathogen contamination in the produce production environment.

Purpose: The purpose of this study was to examine the prevalence, persistence and diversity of *Listeria monocytogenes* in two spinach fields from planting to harvest.

Methods: Spinach fields were sampled nine times over the 48 day crop. A total of 711 samples (486 soil, 162 spinach leaf, 36 drag swab and 27 water samples) were cultured for *L. monocytogenes* and isolates were analyzed by PFGE. Remotely-sensed meteorological factors were evaluated by logistic regression analysis for their association with the likelihood of an *L. monocytogenes* positive sample.

Results: *L. monocytogenes* was detected in 7.2%, 1.8%, 19% and 77% of soil, leaf, drag swab, and water samples, respectively. Analysis of PFGE-types showed persistence in one field, demonstrated by the recovery of *L. monocytogenes* isolates with the same PFGE-type at least three times over the 48 day crop. A high PFGE-type diversity (each D > 0.90) was observed among *L. monocytogenes* isolates from soil, drag swab and water samples. Of the 39 remotely-sensed meteorological factors assessed, average precipitation (within 3 d of sample collection) and average humidity (within 3 d of sample collection) were identified as important meteorological factors increasing the likelihood of an *L. monocytogenes* positive sample. On the other hand, average solar radiation was shown to decrease the likelihood of an *L. monocytogenes* positive sample.

Significance: This information can be used to develop models that predict meteorological conditions when the prevalence of *L. monocytogenes* may be high. These findings will support the development of science-based mitigation strategies for growers, such as modifications to personnel hygiene and or equipment sanitation to limit potential *L. monocytogenes* contamination transfer from field to packinghouse.

mean log reduction).

Significance: The choice of a testing protocol has a great influence on measured hand sanitizer effectiveness. Alcohol-based hand sanitizers are more effective than those based on other antimicrobials for both bacteria and viruses.

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