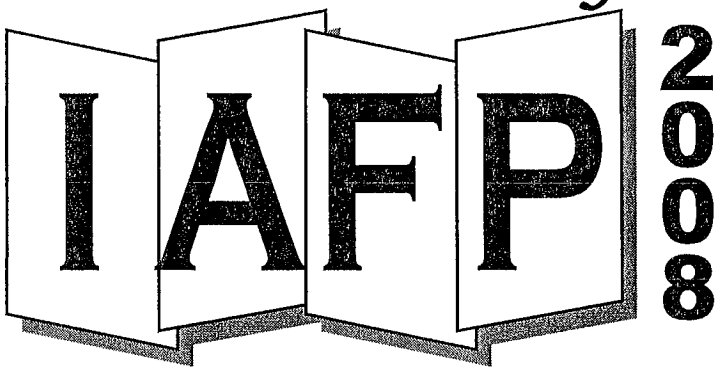
IAFP 2008 ABSTRACTS

95th Annual Meeting



Columbus, Ohio • August 3–6

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OUR MISSION

"To provide food safety professionals worldwide with a forum to exchange information on protecting the food supply"

Ivan Parkin Lecture Abstract

Utility of Microbiological Testing for Food Safety Assurance: The Good, the Bad, and the Ugly

Presented by

Dr. Russell S. Flowers

Silliker Group Corporation
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Microbiological testing is an important component in assuring food safety. It is used to establish baseline information for microorganisms in ingredients, in-process samples, the process environment, and to validate the efficacy of lethal and preservation processes. Microbiological testing also is useful for verification of critical ingredients, the processing environment, critical control points (CCPs), and the overall HACCP plan. Microbiological testing may be required as part of a trade agreement between countries (import/export), or part of a purchase specification between buyer and supplier. In addition, regulatory programs have incorporated microbiological guidelines, such that routine process control testing is required. Some food producers perform routine finished product testing as due diligence to minimize liability in the event of litigation. These different applications of microbiological testing require varying levels of accuracy, precision, sensitivity, specificity, time, and cost constraints. The confidence or uncertainty associated with microbiological data can be affected by various steps in the process; including the sampling plan, sampling procedure, method of analysis, and laboratory performance.

With the exceptions of baseline development and process control, applications of microbiological testing in food safety programs requires that limits be established to differentiate acceptable from an unacceptable product. Although it has been many years since the International Commission on Microbiological Specifications for Foods (ICMSF) provided guidance on development of microbiological criteria, many microbial limits set by industry and government fail to include necessary components that determine the reliability of the data for the purpose intended. Even when these components have been defined, they are often set without a clear understanding of the distribution and state of the target organism(s) in the food. Governments and industry have established zero-tolerance criteria for certain food pathogens, even though no practical sampling plan and testing protocol can assure the absence of a pathogen.

Despite the many inappropriate applications of microbiological testing today, there is hope for the future. Risk-based food safety objectives, combined with improved understanding of microbial distribution and sampling statistics, will result in more meaningful microbiological criteria to be developed. Cumulative across lot and process control testing should allow verification of performance at levels not practical with single lot acceptance testing. Improvements in method validation and laboratory quality programs will provide a better understanding of the uncertainty of analytical data. The combined effect of these developments should greatly enhance the value of microbiological testing for food safety assurance.

JOHN H. SILLIKER LECTURE ABSTRACT

FROM WILD PIGS AND SPINACH TO TILAPIA AND ASIA: THE CHALLENGES OF THE FOOD SAFETY COMMUNITY

PRESENTED BY

DR. MICHAEL P. DOYLE

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Vegetables and fruits have become leading vehicles of illnesses associated with foodborne outbreaks. Fresh-cut produce, which is cut, shredded, diced or peeled, can pose an increased public health risk because this wounded plant tissue enables microbes to more easily attach and grow on the nutrients released from the plant. Presently, neither processors nor consumers have available a treatment that assures the microbiological safety of fresh produce contaminated with large populations of foodborne pathogens prior to treatment. Hence, current production and processing practices cannot be relied upon to ensure pathogen-free fresh and fresh-cut produce. Effective food safety interventions to minimize pathogen contamination are needed for implementation and final preparation of fresh and fresh-cut produce. Currently, approximately 15% of food consumed in the United States is imported. Developing countries are major food exporters to the US and will likely become predominant sources of the US food supply. Fresh produce, fresh and frozen fish and shellfish, and nuts are dominant food groups imported by the US. Food in many countries is not produced under acceptable sanitary practices, and FDA inspects <1% of more than 9 million imported food entries annually. Unless food production, harvesting, and processing practices are upgraded in food exporting countries that have major holes in their food safety nets, there are likely to be increases in the occurrence of foodborne illnesses in the US.

S1 2008 Foodborne Disease Update: *Salmonella* in Processed Foods

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This symposium provides detailed information and discussion on recent foodborne disease outbreaks and their impact on food safety. *Salmonella* Tennessee associated with peanut butter and *Salmonella* 4,5,12:i:- associated with pot pies were both large 2007 outbreaks in the US that have had a significant impact on consumers, the food processing industry and on food safety regulatory agencies. The epidemiology of the outbreaks as well as the environmental investigation and food regulatory responses will be discussed. A discussion of the 1996 peanut butter outbreak in Australia provides the backdrop for the recent US investigation.

S2 Coming Out of the *Campylobacter* Closet: International Strategies for Reducing Human Campylobacteriosis

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The closets of many countries are now being unlocked and opened to expose a far greater incidence of campylobacteriosis than previously notified. New Zealand no longer sits alone at the campylobacteriosis table. The widespread recognition that campylobacteriosis is the primary bacterial gastroenteritis in the developed world is now focusing governments on the need to implement comprehensive risk mitigation strategies.

Previous symposia at IAFP have investigated in depth the disease itself; analytical procedures for selection, isolation and molecular typing of *Campylobacter*; efforts to assign proportionality of transmission routes; and possible antimicrobial interventions. While there have been significant enhancements in molecular typing and source attribution, it is not the intention to of this symposium to address these issues again in depth. After all, none of these topics in isolation will solve the problem of campylobacteriosis.

Instead, this symposium asks the questions, "What are competent regulatory authorities (e.g., government regulators) actually doing to reduce the burden of human campylobacteriosis? What strategies and programmes have they put in place? Do these strategies address issues on-farm, at the processor, during the supply chain and for the consumer?" International government and industry speakers will present the CODEX strategies, and those of individual countries and regions; New Zealand, Scandinavia, Iceland, Canada, the United Kingdom and the United States and openly debate implementation issues.

Most importantly, the symposium will highlight just how successful, or not, these ongoing strategies have been in reducing both the exposure to *Campylobacter* and the burden of human campylobacteriosis. One must ask — are we there yet?

S3 Globalization of Acceptance Criteria for Microbiological Methods: Separating the Science from the Politics

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The purpose of this symposium is to discuss whether or not method harmonization or harmonization of validation protocols are the only approaches for international acceptance of analytical methods. As the world food market continues to grow globally, there is an ongoing need for greater harmonization of regulatory requirements to increase efficiencies in moving safe products across borders. In addition, international food companies must utilize similar testing methodologies in all their facilities, regardless of geographical location. Concurrently, novel methods for microbiological assessment are being developed to reduce turn-around time and enhance analytical efficiency. However, the lack of global harmonization for method validation protocols is a major impediment to the adoption of such novel methods by international regulatory agencies. Even though there is general agreement on the need for validation studies, each method validation organization or regulatory agency has their own unique protocol requirements and designated reference methods. Many of these reference methods or "gold" standards were often written by the subject matter authority at that time and became politically entrenched. The requirements for comparative study against these "standards" often limit the ability of industries and agencies to adopt newer methods, particularly when the newer or more novel methods have better sensitivity, specificity or precision. Such conflicts are challenging to companies that develop proprietary methods and organizations that

conduct or organize collaborative studies to validate their performance characteristics. Attempts to establish USP/EP harmonization for method validation protocols in the pharmaceutical industry has had many unforeseen pitfalls, which necessitated a delay in implementation. A better approach may be the universal acceptance of desirable performance characteristics rather than the focus on validation protocols. As long as you know what you want to achieve, does it matter how you get there? It's time we walked the talk on the evaluation and adoption of methods based on the fit-for-purpose concept. Following the formal presentations, we have a panel to discuss and answer questions about what it will take to eliminate nationalism from microbiological validation protocols so that we can achieve a more science-based, seamless global acceptance process for analytical methods.

S4 Bacterial Physiology — A Forgotten Theme That is Critical for the Food Microbiologist

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While molecular biology and genomics have helped us make tremendous strides in our understanding of food-associated bacteria, as well as in our ability to detect foodborne pathogens, a thorough understanding of the effects of bacterial physiology on bacterial behavior and phenotypes represents a critical, but often overlooked, need. Bacteria grown at different temperatures and oxygen tensions and exposed to different stress conditions as well as bacteria in different growth phases can show significant differences in a variety of phenotypic traits, including their stress resistance, virulence, ability to grow or persist in foods, and ability to survive different inactivation treatments.

S5 Sampling and Sample Prep: Unglamorous but Very Necessary

MARY LOU TORTORELLO, FDA-CFSAN, National Center for Food Safety & Technology, 6502 S. Archer Road, Summit-Argo, IL 60501, USA; BYRON BREHM-STECHER, Iowa State University, Dept. of Food Science and Human Nutrition, Ames, IA 50011, USA; J. EMILIO ESTEBAN, USDA-FSIS-OPHS, 320 Central Ave., Bldg. 2A, Alameda, CA 94501, USA; TONY SHARPE, Filtaflex Ltd., Almonte, ON, K0A 1A0, Canada; MARK CARTER, Silliker Inc., 160 W. Armory Drive, South Holland, IL 60473, USA; DAVID GOLDEN, University of Tennessee, Dept. of Food Science and Technology, 2605 River Drive, Knoxville, TN 37996, USA; LEE-ANN JAYKUS, North Carolina State University, Dept. of Food Science, Box 7624, Raleigh, NC 27695, USA

Sampling and sample preparation are often overlooked aspects of microbiological methods research. Although huge improvements in detection and identification of microorganisms have become available as a result of advances in molecular biology, these methods only work well if target organisms are present in sufficient levels to overcome interferences presented by the food matrix. As a follow-up to the 2005 symposium entitled "Enrichment Media and Sample Preparation: What's New?" this symposium reflects continuing needs and interest in the upstream side of microbiological methodology. It will present the activities of the IAFP Sample Preparation Working Group; sampling and compositing strategies; considerations for quantitative or non-enrichment-based methods; how combining sample processing with rapid detection has been applied in emergency response and outbreak investigation; and other novel approaches for improved sampling and sample processing.

S6 New and Innovative Ways to Derive Risk-Based Management Options

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Codex Alimentarius and governmental agencies around the world together have developed the concept of risk-based food safety management, and are now in the process of issuing "user" guidelines. However, there is a limited practical experience in the world on how to bring the concepts into actual operation and what bottlenecks need to be dealt with. This symposium discusses where in the process to consider what methods can be used to derive risk management options and to set process parameters as an option, both in public and private sectors, by providing examples of valuable practical experiences, and identifies some challenges and opportunities dealing with bottlenecks. This would be helpful to the IAFP community in understanding the progress in this field over recent years and the expected path of future developments.

S7 Food Safety Issues in Food Transportation — Keeping It Cold and Keeping It Clean

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Food transportation (e.g., bulk food transport, finished product distribution) presents a diverse variety of food protection challenges which impact shelf life, food safety, and food defense. Maintenance of proper temperatures during shipment from suppliers to distributors is the key to preserving product integrity, quality, and safety. It is significantly important to understand the importance of using reputable distributors, monitoring products during transport and how they can be employed to maintain integrity and to trace sources of mishandling and abuse. The use of high functional sensor equipped RFID tags can significantly improve the food quality, safety, security and traceability. A variety of bulk liquid and dry food products (e.g., milk, juice, vegetable oils, sugars, dry milk, other products) are transported in over-the-road tankers or other conveyances. Many of these vessels and conveyances are not fabricated from stainless steel, nor under 3-A specifications. In addition, bioterrorism and product tampering concerns have heightened the need for effective security protection, as well as traceability during transport.

Participants will come away from this symposium with effective methods to mitigate safety risks during bulk food shipment, as well as final product distribution, through enhanced sanitation, security and traceability.

S8 Validating Processes for Reducing *Salmonella* in Low Water Activity Foods

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In recent years there has been a number of salmonellosis outbreaks linked to the consumption of low water activity foods including white and dark chocolate, peanut butter, almonds, savory snacks, and cereal products. It has been documented that reduced water activity and other factors can lead to increased resistance in *Salmonella* spp. This phenomenon is a critical consideration when designing and validating processes for the thermal and non-thermal inactivation of this pathogen in foods. This symposium will examine those factors that affect the resistance of *Salmonella*, key considerations when determining D-values for this organism in specific matrices, the role of surrogates in validating thermal inactivation processes, and provide a real world case study on translating laboratory data to equipment validation. Issues will be examined and recommendations provided on coherent approaches to validating processes for the reduction of *Salmonella* in low water activity food products.

S9 Advancements in Retail Food Safety

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Food safety awareness is at an all time high, new and emerging threats to the food supply are being recognized, and consumers are eating more and more meals prepared outside of the home. Accordingly, retail and foodservice establishments have a growing responsibility to ensure that proper food safety practices are followed.

Achieving food safety success in this changing environment requires retailers and foodservice establishments to go beyond traditional training, testing, and inspectional approaches to managing risks. Come hear from leading experts about new advancements in retail food safety.

S10 From Fish to Table

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Consumption of seafood has increased worldwide and now has caused food safety concerns. In the United States during 2007 consumption rose to 16.6 lbs per person with 40% obtained from aquaculture operations and during the 10 year period (1983–1993), finfish was the third most reported vehicle of transmission, causing 18% of the total number of foodborne illness outbreaks. This symposium will cover various food safety aspects of delivering farm raised finfish to the consumer. Speakers will discuss the risk/benefit of eating fish as it relates to environmental pollutants (mercury and PCBs) which can accumulate in selected seafood products; and human pathogens (bacteria and viral) and parasites on the raw finfish. An overview of food safety concerns during harvesting and filleting of finfish will be provided, and representatives of the restaurant and retail industry will discuss the food safety issues of raw fish during post harvest handling, shipping, packaging and sale. Insight will be provided on education for the safe handling of raw fish and on the impact of consumer safety in the home.

S11 Best Practices in Global Food Export and Import

ROBERT L. BUCHANAN, FDA-CFSAN, HFS-06, 5100 Paint Branch Pkwy. #2B64, College Park, MD 20740, USA; WOLF MAIER, European Commission Delegation, 2300 M St. NW, Washington, D.C. 20037, USA; JOHN BASSETT, Unilever, SEAC, Colworth Park, Sharnbrook, Bedford MK44 1LQ, England; SUELY M.K. NAKASHIMA, Sadia Foods GmbH, Lyoner Strasse, 44-48, Frankfurt, Hessen D-60528, Germany; CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, 1875 Connecticut Ave. NW, Suite 300, Washington, D.C. 20009, USA; JOHN W. SPINK, Michigan State University, 1835 Yosemite Drive, Okemos, MI 48864, USA

Agricultural and food trade throughout the world is changing. Keeping up with fluctuations in markets, new and amended regulations, and emerging foodborne

hazards that affect trade, is challenging. Technology and better market access through the WTO's attempts at lowering non-tariff barriers to trade have opened international markets for both buyers and sellers. Finding new markets overseas, expanding production, stabilizing food supply and improving the safety of foodstuffs are just a few of the benefits of international food trade. But safeguarding the public health in an era of international trade does not just happen. This symposium aims to give an overview on internationally established and regularly implemented procedures with regard to world food commerce. There will be international speakers covering topics on equivalence and international comparison of food safety systems, food trade regulations from an EU standpoint, issues and policies with respect to traceability and sourcing as applicable to multinationals' import activities, and international food export with particular emphasis from a Brazilian perspective. From the US side, there will be talks on the impact of imported foods and labeling of such food products on consumer acceptance, and the recognition that illegal global food trade activities such as smuggling and counterfeiting of imported food are a part of the food supply.

S12 Back to the Future: How Clinical Microbiology Findings Today Predict the Food Microbiology Headaches for Tomorrow

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Food safety is an evolving science with an increasingly sophisticated toolbox of techniques for pathogen detection. This symposium aims to show the predictive side of food microbiology and how future needs for pathogen detection in foods can be anticipated from new findings in epidemiology and clinical medicine. This will be demonstrated on a continuum – from the past with established pathogens (STEC, Hepatitis A) to the present with an evolving pathogen (noroviruses, hepatitis E) to the future with possible foodborne pathogens (*Clostridium difficile)* or diseases with a potentially infectious foodborne etiology (irritable bowel disease).

S13 Pathogen Data Sharing to Advance Food Safety

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An understanding of where pathogens enter the food supply chain, where they multiply/die/survive, how they impact human health, and how new controls can be implemented is critical for risk analysts in both the public and private sectors. The linkage to human health is especially critical, since food safety is the aim of pathogen control programs. This session discusses the current status of US and international data sharing among government agencies, the private sector, and consumers. Challenges to better data sharing may include the wide array of tests used, legal statutes, and leadership in the public and private sectors. This session analyses the problems, points to successes, and helps shed light on data sharing in this new era of pathogen testing throughout the food chain. Identification of food/product/company linkages that are causing foodborne disease, both sporadic cases and outbreaks, is important for several reasons: to increase economic incentives for companies to provide safer food, to improve the ability of regulators to discover new patterns of foodborne illness and set priorities, and to enable consumers to understand foodborne risks and modify their behaviors re food consumption and preparation as well as legal actions when foodborne illness strikes.

S14 Food Safety and Regulatory Issues Associated with Non-Thermal Processing of Foods and Beverages

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Recent innovations in non-thermal food processing offer certain quality advantages over thermal processing or chemical preservatives for foods and beverages. The non-thermal processing technologies with most commercial promise include: removal of bacteria using filtration or centrifugation, use of electrical energy (pulsed electric field, ohmic heating), use of high pressure and high pressure CO_2, and use of radiant energy. Recent recommendations by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), the requirements under the US FDA's Juice HACCP regulations, and the Federal Food, Drug and Cosmetic Act (FDCA) definition of pasteurization, are written such that they do not prohibit the use of effective non-thermal processing methods. However, the pasteurization definition for fluid milk and milk products under the Pasteurized Milk Ordinance (PMO) is slightly more restrictive in that, in addition to specifying the temperature and time (or equivalent), it requires the milk be processed in properly designed, calibrated and operated equipment which is routinely inspected by the regulatory agency. Since microbiological destruction kinetics of non-thermal processing are not as well understood as those for thermal destruction, validation of the effectiveness of non-thermal processes may present a challenge under HACCP and/or other regulations. Ionizing radiation, defined under FDCA as a food additive, is a special case in that it is approved on a case-by-case basis by FDA. In this symposium, examples of alternative processing technologies will be discussed with special emphasis on their technical aspects, effectiveness in pathogen reduction, commercial feasibility, consumer perceptions and potential regulatory implications.

S15 Harmonization of Irrigation Water Practices

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The safety and quality of fresh fruits and vegetables and fresh-cut produce has been in the headlines and captured the public's attention. As risk-based approaches begin to evaluate factors in and near the growing fields, which could contribute to potential contamination of produce, one variable that can greatly impact the safety of fresh fruits and vegetables is irrigation water quality. The IAFP's Fruit and Vegetable Safety and Quality Professional Development Group (PDG) and the Water Safety & Quality PDG joined together to present a symposium that focuses on irrigation water practices.

Harmonization of irrigation water practices has gone unaddressed in the food industry. While issues of concern have been raised in the past, recent events involving irrigation waters have raised the level of awareness of its importance considering the various practices that are used today in different regions of the US and around the world.

This symposium will take a look at what constitutes irrigation waters in different regions and what, if any, regulations governs their source, amount, quality, use and/or reuse. National and international perspectives will be discussed due to the globalization of the food supply chain.

The microbiological issues relating to irrigation water safety play an important role in the discussion of harmonization. Non microbial threats will be examined and must be given as much consideration as the biological threats, for these threats can pose long term problems and they may not be easy to eradicate cost effectively.

Animal agriculture's impact on irrigation water quality will be discussed. As will case studies from US growers in the fresh fruit and produce community who will explore what has and has not been viable for farmers that rely on these waters to produce their crops year after year.

The Water Safety & Quality and Fruits and Vegetable Products PDG's hope this symposium will start the dialogue to see how harmonization of irrigation practices can begin to play an important role for global food safety.

S16 Spores in the Dairy Industry — A Growing Concern — What Can You Do?

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Spore-forming bacteria have developed into a significant problem in dairy plants over the last decade. The bacteria and spores they form have caused end of code shelf-life issues in fluid milk plants and is known to cause problems in cheese plants and powdered dairy operations. The issue has risen significantly as a cause of problems in the last 5 years and now shows up regularly in plant troubleshooting. This symposium will address what the state of the industry is, what the current research is showing and how industry (farm and plant) can address the problem using current technology.

Symposium

S17 Dairy Pasteurization in Today's Risk-Based Food Safety Environment — International Perspectives on the Use of Risk Assessment Tools

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Raw milk is inevitably contaminated with a range of bacteria that include human and animal pathogens. There is no doubt that pasteurization of milk has controlled these hazards and its benefit to public health is unquestioned. However, in today's risk-based food safety environment it is inevitable that the question is asked as to whether the level of hazard control achieved by pasteurization is more than necessary to mitigate the risk of milkborne pathogens? Can lower time and temperature combinations or alternative non-thermal processes be used and still achieve an appropriate level of consumer protection in today's dairy environment?

Internationally, there is much interest in answering these questions, and microbiological quantitative risk assessment (QRA) is being used by several competent authorities (e.g., government regulators) towards this end. This symposium describes the use, advantages and pitfalls, of QRA for reassessing the risk-basis of pasteurization, and describes studies to fill identified data gaps; a major example of which is kinetic thermal inactivation data for milk-borne hazards under modern commercial heat treatment conditions. The symposium will present the results of extensive process-based studies to fill this data gap.

However, while the ultimate goal of QRAs is to aid in the making of risk management decisions, many non-scientific factors, e.g. societal and cultural values, influence the risk management process. The symposium will describe how several countries, New Zealand, Australia, France, Canada and the United States have incorporated such influences into their risk management decisions for milk products, and open the floor to lively debate.

S18 Innovative Applications of Bacteriophages in Rapid Enrichment, Detection and Identification of Foodborne Pathogens

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Rapid detection and identification of foodborne pathogens continues to be an integrated part of food safety and quality assurance program. In recent years, technologies using bacteriophages for the rapid methods have advanced dramatically.

These technical applications include: (1) Bacteriophages specific to growth competitors of target pathogens were isolated and added in media as selective agents during enrichment, which allows high productivity of pathogens and increases the overall assay specificity. (2) Whole phages specific to *Salmonella* group are used to infect enriched samples, and the lytic *Salmonella* cells release adenylate kinase, which facilitates the bioluminescence detection. (3) Proteins from pathogens-specific bacteriophages were also prepared and coupled to magnetic beads for sample preparation before detection. This sample preparation method has been combined with PCR-based and automated enzyme linked fluorescent assays, which result in an increased sensitivity at a significant reduced total detection time for *Listeria, E. coli* O157 and *Salmonella*. (4) Antibody fragments specific to *Listeria* were selected via phage display and used successfully for the developments of immunoreagents for pathogen detection. (5) Specific bacteriophages are introduced to the processed sample, where they find, infect, and amplify their target pathogen(s) providing a very specific, highly amplified surrogate marker for rapid detection and identification.

Some of these applications have been successfully commercialized. This symposium will review and update the scientific knowledge in this area and promote more research and application interests into future.

S19 Chemical Contaminants Testing in Foods

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The risk associated with chemical contaminants in foods has received a great deal of concern in recent years. Chemical contamination of foods may occur through environmental pollution (e.g., toxic metals, PCBs, perchlorate and dioxins), the intentional use of various chemicals (e.g., pesticides, animal drugs and other agrochemicals), or the migration of chemicals

from packaging materials. It may also occur as a result of food processing, such as the case with heterocyclic amine, acrylamide and furan. In response to the increased concern, the food industry and regulatory agencies have been vigilant in the monitoring of hazardous chemical residues in food. Advances have been made in the development of accurate and cost-effective methods for the analysis of many of the chemical contaminants in foods. Low-cost, high throughput assays have been developed to allow screening of multi-residues using a single procedure. This symposium will highlight current issues relating to the chemical contaminants in foods and provide an overview of the methodologies used for the detection and testing of chemical hazards in various food matrices. New technologies as well as international standards for the analysis of emerging chemical hazards such as veterinary drug residues in milk and dairy foods, antibiotics in seafood, pesticide residues in produce, and toxic chemicals formed during thermal processing of foods will be discussed.

S20 Food Defense Educational Programs and Opportunities Status: Focus and Future

SHAUN KENNEDY, National Center for Food Protection and Defense, University of Minnesota, St. Paul, MN 55108 USA: DAVID MCSWANE, School of Public and Environmental Affairs, Indiana University, 107 S. Indiana Ave., Bloomington, IN 46202, USA; CRAIG HEDBURG, School of Public Health, University of Minnesota, 420 Delaware St. SE, Minneapolis, MN 55455, USA; ABBEY NUTSCH, National Agricultural Biosecurity Center/Food Science Institute, Kansas State University, 203 Fairchild Hall, Manhattan, KS 66506, USA

Since September 11, 2001, a heightened awareness has developed regarding the security and defense of global agriculture and food supplies against intentional acts of sabotage. Governments and industry have recognized the profound risks that exist and the vulnerabilities associated with this critical infrastructure and have reacted by issuing policies, guidelines, training materials, and production/manufacturing programs to help secure food systems. As our understanding of these risks improves, and as food protection approaches evolve and mature, the importance of formal educational programs to train personnel focused on food defense is magnified. This symposium will evaluate and define the need for such educational programs, assess approaches to effectively developing appropriate university-based curricula, and highlight degree and certificate programs in food defense currently being offered by universities in the United States. The symposium will conclude with a panel discussion (with audience participation) on gaps, approaches and future.

S21 Is It Overdone? Examining the Meat and Cancer Hypothesis and Its Impact on Food Safety

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This symposium aims to stimulate scientific dialog on the relatively well-established food safety practices used to ensure microbiologically safe meat and poultry products (e.g., thermal processing, nitrite curing) contrasted against the potentially conflicting recommendations that are offered to address theoretical risks of the "meat and cancer hypothesis." Red meat and processed meat products are often dietary components targeted for investigation in nutritional epidemiology studies assessing the potential link between diet and human cancer. For most forms of cancer, there has been no established positive association related to meat consumption. However, in some studies red meat and processed meat have been epidemiologically associated with certain forms of cancer. Typically the associations reported are weak with odds ratios falling below 1.5 and frequently not statistically significant. Some studies have suggested mechanisms for the potential effect being observed in the dietary surveys. While a recent Systematic Literature Review (WCRF 2007) indicated that there were "no plausible biological mechanisms operating in humans with respect to meat consumption", some reports allege that certain compounds used in meat preparation (e.g. nitrite) or produced in meat during cooking (e.g. heterocyclic amines) may be causative agents in cancer development and promotion. The "meat and cancer hypothesis" has led some to suggest that changes in meat preparation must be taken to minimize this theoretical risk. However, changes being recommended may have a negative impact on meat safety. Proper time and temperature during cooking is required to achieve microbiological safety; however it is suggested that high temperature and or long time cooking can lead to formation of potentially carcinogenic heterocyclic amines. Nitrite has been used for decades as an effective means of preventing outgrowth of *Clostridium botulinum*, yet some suggest this compound is linked to negative health outcomes. However, the National Toxicology Program in 2000 found nitrite did not cause cancer in laboratory animals and new research has suggested that dietary nitrite may play an important role in cardiovascular health. These examples and others will be used to discuss how different forms of scientific evidence should be reviewed and how the risks and benefits should be considered prior to making dietary recommendations about risk reduction.

S22 What is the "Real" Issue with MDR?

SHAOHUA ZHAO, FDA, Center for Veterinary Research, 8401 Muirkirk Road, MOD 2, Laurel, MD 20708, USA; PAULA FEDORKA-CRAY, USDA–ARS, 950 College Station Road, Athens, GA 30605, USA; JEAN WHICHARD, CDC, 1600 Clifton Road, Atlanta, GA 30333, USA; JOHN THRELFALL, Health Protection Agency, 61 Colindale Ave., London, NW9 5EQ, UK

Since the early 1990s there has been increasing awareness and concern regarding the development of antimicrobial resistance among bacteria of public health significance. Reports targeting zoonotic bacteria, and in particular *Salmonella* species, suggest that multiple drug resistant (MDR) is trending upward. In the UK, MDR *Salmonella* Typhimurium DT104 exploded and reached epidemic proportions. DT104 was unique in that it exhibited a penta-resistant pattern of resistance to Ampicillin, Chloramphenicol, Streptomycin, Sulfamethoxazole, and Tetracycline (ACSSuT) which was chromosomally integrated. Cattle appeared to be the primary reservoir in animals although a wide-range of animals presented with DT104. Contaminated foodstuffs were responsible for severe outbreaks and increased morbidity and mortality. In the US, DT104

also emerged, but has been primarily concentrated in cattle from the Pacific Northwest. Although attributed to several outbreaks, DT104 in the US has never been associated with the extent of morbidity and mortality observed in the UK. However, starting in 2000, the emergence of multiple drug resistant (MDR) *Salmonella* Newport was the focus of much research. Like DT104, *S.* Newport is often isolated from cattle although in recent years an increase in isolation from chickens has been observed. Unlike DT104, resistance is plasmid mediated and has been associated with a number of foodborne outbreaks. Interestingly, in recent years both DT104 and Newport have decreased in incidence while other MDR serotypes such as Agona, Reading, and Heidelberg appear to be increasing. Further, MDR is not only confined to *Salmonella* as MDR is also observed in other foodborne pathogens including *Campylobacter* as well as the commensal bacteria *E. coli* and enterococci.

The purpose of this symposium is to present the current knowledge and status of MDR both here and abroad. The speakers will present the mechanism of MDR, describe the ease of which MDR can spread, and provide an update on the MDR plasmid that is increasing in prevalence. An update on MDR in foodborne and commensal bacteria in the US will focus on the status in animals and humans and the complement will be presented from 'across the pond'. Finally, the speakers will reconvene as a panel to engage the audience. Following this symposium, attendees will have a better perspective on what drives MDR as well as the status of MDR in foodborne bacteria both here and abroad.

S23 The Greening of Food Packaging — Safety of Biodegradable, Reused and Recycled Food Packaging

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The growing global awareness regarding sustainability and the overuse of energy resources by the western industrialized countries, the significant increase in the cost of energy to produce food packaging materials and the huge amounts of food packaging material that ends up as waste have revitalized interest by consumers, the food industry and governments to reduce and recycle food packaging material or moving to more biodegradable alternatives. Significant issues related to the technology and safety of recycled and biodegradable food packaging material in direct contact with food has in the past prevented this "green" philosophy from being widely adopted. This symposium will:

- address the new technologies related to eduction, recycling and conversion to biode gradable alternatives to existing food packaging materials.
- investigate the food safety implications related to recycled and bio-degradable food packaging, and
- evaluate the regulatory roadblocks in utilizing available new technologies by the food packaging industry.

S24 Food Allergens: Scientific Advances and Control Measures

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Food allergy affects more than 11 million people in the US and the incidence seems to be increasing. Some of the affected individuals can develop life-threatening reactions after ingestion of a minute amount of allergenic foods. Strict avoidance of the offending food is the only successful method to prevent the occurrence of allergic reactions. The major focus in managing food allergy risk by the industry and the regulatory agencies is, therefore, to prevent the inadvertent exposure of sensitive individuals to the allergenic foods. Increasingly food manufacturers have implemented improved allergen controls measures to prevent such occurrences. Regulatory agencies have developed regulations and policies to require labeling of allergenic ingredients. The underlying mechanism of food allergy is not well understood. However, an increasing body of basic and clinical research has been performed to gain insight to the mechanism of food allergy and to develop innovative therapies. Advances have also been made in the identification and characterization of allergenic proteins in food in an attempt to understand what establishes a protein as an allergen. This symposium will provide an overview of the scientific advances that have been made in recent years with respect to food allergy and food allergens. It will also review the control measures that have been put in place globally. The presentations will cover the allergen issues from the medical, manufacturing, regulatory and international trade perspective, with a focus on international standards and their impact on food industry.

Roundtable Abstracts

RT1 Eating Seafood — Is It Worth the Risk?

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Deficiencies in the FDA's Fish and Fisheries Products – Hazards and Control Guidance (3rd Edition) were identified. Some of the questions of concern were the cooking requirements for the different seafood types, e.g., finfish, shellfish or crustaceans to eliminate potential pathogens, consumer education on safe handling and cooking of various seafood, and research needs to determine the cooking conditions and seafood preparation. This roundtable will focus on these questions. Panel speakers will share their insight on the cooking requirements for the different types of seafood, the consumer's concern of pathogenic bacteria and viruses and parasites in the seafood they purchase, research and consumer education needs for safe cooking and handling of seafood by the consumer. Audience participation will begin with questioners who have expertise in the areas of nutritional education, retail and marketing and the food industry.

RT2 Occurrence and Control of Norovirus: Is Public Vomiting Public Enemy #1?

MELVIN KRAMER, EHA Consulting Group, Inc., 7904 Starburst Drive, Baltimore, MD 21208, USA; JAN VINJE, CDC, 1600 Clifton Road NE, Atlanta, GA 30333, USA; DAVID BROWN, Health Protection Agency Colindale, Centre for Infections, 61 Colindale Ave., London NW9 5EQ, UK; HAL KING, Chick-fil-A Restaurants, 5200 Buffington Road, Atlanta, GA 30349, USA; CHARLES GERBA, University of Arizona, 429 Shantz Bldg., Tucson, AZ 85721, USA

Occurrence and control of foodborne viruses is a significant public health issue. Human noroviruses are food-and waterborne pathogens of great importance. Recent detection of human noroviruses in a variety of foods and the emergence of recombinant strains with heightened virulence increases the need for good epidemiology and control measures. These issues impact public health worldwide as evident by outbreaks in Europe and the United States. Trends that have been identified by CaliciNet in the European Union will be discussed in this symposium along with their impact across the Atlantic. Transmission occurs from person-to-person contact and from contaminated foods as well as from fomites. Control measures must be applied at multiple levels, and there is a great need to understand the survival under various conditions in order to develop appropriate controls. During this symposium current trends in epidemiology and detection will be discussed along with their impact in outbreak detection and control at varying levels. Expert viewpoints from government, academia, and industry will be provided. The panel roundtable discussion will provide an opportunity for the audience to address the experts with their concerns about occurrence and control of noroviruses and other enteric viruses. Additionally the panel will help implant the concept of a science-based protocol for retail food establishments to consider in the event of a public vomiting and/or diarrheal event in a retail food establishment. This issue has emerged with increased importance following several large outbreaks this past year.

RT3 Does Internalization of Pathogens Occur in Fresh Produce during Commercial Production and Processing?

KARL MATTHEWS, Rutgers, The State University of New Jersey, 65 Dudley Road, School of Env & Bio Sciences, New Brunswick, NJ 08901, USA; KEITH WARRINER, University of Guelph, Dept. of Food Science, Bldg. 038, Guelph, Ontario N1G 2W1, Canada; JOSEPH FRANK, University of Georgia, Center for Food Safety, Athens, GA 30602-7610, USA; LARRY BEUCHAT, University of Georgia, Center for Food Safety, Griffin, GA 30223-1797, USA

Laboratory-based experimentation suggests that bacterial species such as *Salmonella enterica* or *Escherichia coli* O157:H7 can invade the vascular systems of growing plants or tissues damaged during post-harvest treatments or processing. Internalization is presumed to shelter human pathogens from environmental stresses incurred during production or during postharvest disinfection treatments and fresh-cut processing. Hence internalization has serious implications for the safety of fresh produce and the risk of this occurrence in commercial production and processing systems must be understood. The symposium panel will consist of four scientists with extensive research experience in the field to summarize the current state of knowledge and to discuss the implications of pathogen internalization on agronomic practices during production, the design of disinfection treatments and the sampling and analysis of fresh produce. Brief presentations by each panelist will be followed by audience questions and roundtable discussions.

RT4 Global Perspectives and Novel Approaches for Effective Food Safety Communication within Culturally Diverse Audiences

VINCENT FASONE, Columbus Public Health, 240 Parsons Ave., Columbus, OH 43215, USA; DAVID MCCLEERY, Food Safety Promotion Board, 7 Eastgate Ave., Eastgate, Cork, Ireland; PHILPPA ROSS-JAMES, New Zealand Food Safety Authority, South Tower, 86 Jervois Quay, Wellington, New Zealand; HELEN YU, Asian Food Information Centre, P.O. Box 140, Phrakanong Post Office, Bangkok, 10110, Thailand

Effective communication of food-safety risks, expectations and responsibilities is a necessary step in

reducing the global burden of foodborne illness. Food handlers from farm-to-fork are key players in impacting public health, and in this age of globalization, many are working in new situations where standards and customs are foreign to them. Differing languages, cultures and traditions provide hurdles to communicating food safety messages to food handlers and have become a barrier to implementing good food safety practices. Innovative methods for acquiring risk information and designing messages must be developed to overcome these global obstacles.

This symposium will focus on providing the latest strategies for risk management and communication activities for culturally diverse audiences, culled from international examples. The topic will be addressed through a roundtable format to stimulate discussion around novel education tools that have been successful throughout the world. Drawing on experiences and challenges faced by a multinational food service organization, Catherine Adams of McDonalds will moderate the session. Each of the four participants will provide a brief summary of the activities they have undertaken to communicate with various cultures. This discussion aims to help reduce the gaps between food safety science, social science and risk reduction strategies with food handlers of differing cultural backgrounds within the risk analysis paradigm. This discussion is also intended to stimulate conversation among the IAFP membership and encourage effective global food safety educational programs.

RT5 Comparative International Approaches to Regulating Unsafe Food

CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, 1875 Connecticut Ave. NW, Suite 300, Washington, D.C. 20009, USA; DEON MAHONEY, Food Standards Australia New Zealand, P.O. Box 7186, Canberra, ACT, Australia; PAUL B. YOUNG, Waters Corporation, Atlas Park, Simonsway, Manchester, England M22 5PP, UK

This session will provide a comparative perspective of the legal definitions and interpretations of adulteration/unsafe food in four regions. Speakers from the US, Europe, Japan and Australia will discuss the approaches taken in those countries or regions. The formal presentations will be followed by a roundtable where expert questioners will lead a discussion on how scientific evidence can be used to clarify the concepts of adulteration and unsafe food.

Efforts to harmonize food standards require an understanding of the underlying legal structures used in different countries. This is important from the perspective of managing public health and safety, and in the facilitation of trade. The speakers will describe how adulteration/unsafe food fit into the legal framework in each country and how it is used in risk management, e.g. zero tolerance.

RT6 Water: Potability vs. Drinkability

DAVID BENNITZ, Health Canada, 9th Floor, AL 4909C, 269 Laurier Ave. West, Ottawa, ON K1A 0L3, Canada; JOSEPH A. COTRUVO, Water, Environment and Public Health, 5015 46th St. NW, Washington, D.C., 20016, USA; KATHLEEN T. RAJKOWSKI, USDA-ARS-ERRC-FSITRU, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; JENNIFER BEST, EPA, Office of Ground Water and Drinking Water, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, USA; KEVIN M. MORLEY, American Water Works Association, 1300 Eye St., Suite 701W, Washington, D.C. 20005, USA

There is broad consensus among food growers, processors, public health officials and consumers that water quality fundamentally affects the safety and quality of food products along the entire food supply chain, farm to fork. Yet, the quality standard for water in food is often defined in imprecise terms. For example, potable water can be defined as:

- water that is safe and fit for human consumption[1]
- raw or treated water that is considered safe to drink[2]
- water suitable for drinking or cooking purposes from both health and aesthetic considerations[3]

In the US, jurisdiction over water quality for food processing or in food service operations is a complex matrix, shared by various government agencies. Food safety personnel have generally focused on making sure water is "potable", assuring the water used in food is free from microbial dangers; yet often overlooking chemical and heavy metal hazards.

Food safety HACCP plans, whether they are with food processors, foodservice operations or governments, are designed to prevent contamination of food by water including water that is an ingredient in the food. There is no clear agreement of what water quality and safety standard should apply when water is used for different purposes along the food continuum.

The IAFP's Water Safety & Quality PDG will explore in this roundtable ("Potability vs. Drinkability") what is meant by "potable" water in the food chain. We will have speakers that will examine the US vs. International Potability standards as well as the legal vs. non-legal impact of potable water on food safety. Speakers will provide real world examples of where Certificates of Potability were obtained but fell short in meeting food safety needs. A speaker from the US water utilities will give examples of what water testing is done to public water supplies at the treatment plant to see how that matches up with the need of foodservice or food manufacturing needs. A lively discussion will follow and the PDG plans to write a white paper from the findings of the roundtable discussion.

1. Canadian Guidelines for Food Processing during Adverse Water Events (11/20/2006).
2. Guide to Environmental Issues: Glossary of Terms & Acronyms: Office of Enforcement and Compliance Assurance, U.S. Environmental Protection Agency.
3. Ecoview Glossary: Office of Environmental Information: State of Texas.

SYMPOSIUM SERIES ON FOOD MICROBIOLOGY

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IN CONJUNCTION WITH THE

INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION 95TH ANNUAL MEETING

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 catalytic role in identifying and addressing scientific questions on nutrition, food safety, and consumer health care.

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

S4 Bacterial Physiology – A Forgotten Theme That is Critical for the Food Microbiologist

While molecular biology and genomics have helped us make tremendous strides in our understanding of food-associated bacteria, as well as in our ability to detect foodborne pathogens, a thorough understanding of the effects of bacterial physiology on bacterial behavior and phenotypes represents a critical, but often overlooked, need. Bacteria grown at different temperatures and oxygen tensions and exposed to different stress conditions as well as bacteria in different growth phases can show significant differences in a variety of phenotypic traits, including their stress resistance, virulence, ability to grow or persist in foods, and ability to survive different inactivation treatments.

Genomics Meets Physiology: What Have Genomics Taught Us about the Effects of Growth Phase and Stress Exposure on Bacterial Physiology?

CHARLES W. KASPAR, Dept. of Bacteriology, Food Research Institute, University of Wisconsin, Madison, WI 53706, USA

Genomics is the study of an organism's entire genome. The field of genomics began when Frederick Sanger and colleagues determined the complete nucleic acid sequence of bacteriophage ϕX174 genome. The compact genome was comprised of 5,386 nucleotides. There is still a major thrust on sequencing genomes of various organisms, including humans, but the sequence data from microbial genomes has permitted comparative genome anlayses that highlight the contribution of horizontal gene transfer in the evolution of microbial pathogens. Moreover, the large databases of genome sequence data enable powerful new techniques to help discern gene function, expression, and networks. Microarrays and bioinformatics are two important tools used in these studies. This talk will provide a brief overview of how genomics has advanced, and in some cases misdirected, our understanding of microbial stress physiology. The RpoS and H-NS regulons will be used as examples of microbial networks and their roles in the regulation of microbial physiology and stress protection. In addition, the use of microarrays in studies of acid tolerance will be discussed along with the limitations of data and the need for confirmatory studies of postulated networks and physiological processes, particularly when addressing complex phenotypes. Lastly, the future role of genomics in unraveling microbial physiology and stress tolerance will be presented.

Effects of Growth Phases, Temperature and Stress Exposure on Foodborne Pathogen Virulence: The *Listeria monocytogenes* Example

KATHRYN J. BOOR, Professor and Chair, Food Science Dept., Stocking Hall, Cornell University, Ithaca, NY 14853, USA

Multiple lines of evidence indicate that environmental stress exposure can affect *Listeria monocytogenes* growth and virulence-associated characteristics. The effects of temperature (7 or 37°C), pH (5.5. or 7.4), presence of salt and organic acids (375 mM NaCl; 8.45 mM sodium diacetate [SD]; 275 mM sodium lactate [SL]; or a combination of NaCl, SD and SL) and deletion of *sigB*, which encodes a key stress response regulator, on ability of *L. monocytogenes* to grow, invade Caco-2 cells, and survive exposure to

L. monocytogenes log-phase generation times and maximum cell numbers were not dependent on the alternative sigma factor sigma B in the presence of NaCl and organic acids at concentrations typically found in foods; growth inhibition of *L. monocytogenes* through addition of organic acids was pH dependent; the ability of *L. monocytogenes* to invade Caco-2 cells was affected by growth phase, temperature and presence of salt and organic acids, with the highest relative invasion capabilities observed for cells grown with SL or NaCl at 37°C and pH 7.4; growth of *L. monocytogenes* in the presence of NaCl, SD or SL reduced its ability to survive exposure to gastric fluid; and exposure of *L. monocytogenes* to gastric fluid reduced the enhanced invasiveness caused by growth in the presence of NaCl or SL. These findings suggest that virulence-associated characteristics that determine *L. monocytogenes* infectious dose are likely to be affected by food-specific properties (e.g., pH, presence of salt or organic acid).

ILSI Symposium

Effects of Growth Phases, Temperature and Stress Exposure on Foodborne Pathogen Survival and Stress Resistance: The *Salmonella* Example

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Salmonella is a well-known, widely-distributed organism capable of causing food poisoning, and often found in a wide range of different food products. The organism is not particularly resistant to heat and cannot grow at low pH or water activity values; it also is not able to grow at good refrigeration temperatures. So, one may ask, how does such an organism remain such a problem in our foods? *Salmonella* can be widely distributed in nature, being found in foods derived from animals and plants (usually from animal contamination). Its characteristics are such that it survives stresses placed upon it, and when in conditions that enable it to grow, it can move through lag phase quickly and develop at a growth rate that can at least match, if not overtake, most competing flora. Of course, *Salmonella* does not need to grow to be a problem in foods. In some outbreaks, the infective dose would appear to be low, so the ability of the organism simply to survive in adverse conditions is enough for it to become a problem. This paper will explore issues relating to survival of *Salmonella* under various stresses, and some recent work considering its ability to exit lag phase as observed using genomic based techniques.

Effects of Stress Exposure on Foodborne Pathogen Physiology: The *E. coli* Example

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Escherichia coli are genetically diverse and have evolved the ability to acclimate to and survive in many adverse secondary environments, such as the farm environment and in natural water sources. The ability to adapt to environmental stresses is critical for survival in external environments and for subsequent reentry into the host. Massive reprogramming of the cell, which occurs during adaptation to stress, is reflected in the form of altered gene expression patterns, e.g., genetically identical *E. coli* exposed to different stress conditions may differ in transcript levels of as many as 1,000 genes. Characterizing the adaptation phase at the level of gene expression permits detection of signaling pathways and regulatory cascades that allow adaptation to occur. The transcriptional responses during adaptation to single stresses, including low pH and high osmolarity, have been described for non-pathogenic *E. coli*. However, multiple stresses are used in the food industries to help control *E. coli*, and the response to multiple stresses remains largely unstudied, particularly in *E. coli* O157:H7. This presentation will review recent studies examining the transcriptional changes that occur during adaptation to single and multiple environmental stresses in non-pathogenic *E. coli* and *E. coli* O157:H7. Understanding the global responses to stressors will allow us to understand better how exposure to one stress condition may affect subsequent survival to other stresses, including those imposed by human hosts. Characterization of stress responses could lead to selection and development of prudent strategies for inhibiting growth and survival of pathogens in foods, such as blocking stress signaling pathways.

How the Physiological State of the Challenge Inoculum Affects Validation Study Outcomes

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Challenge tests can be useful for determining the ability of microorganisms to survive and grow in foods and beverages and to determine the effects of process treatments on inactivation. Validation of the effectiveness of conditions necessary to prevent growth of microorganisms and of processes intended to be lethal, however, can only be achieved if considerations are given to the physiological state of cells used in challenge inocula. Conditions to which cells are exposed preceding inoculation are known to affect their level of sensitivity to adverse environments imposed by foods and to treatments and processes applied for the purpose of controlling growth or causing inactivation. The pH, nutrient availability, and a_w of the growth medium, temperature of incubation, and physiological age of cells are some of the factors that can influence survival, growth, and virulence characteristics of cells in challenge inocula. Sensitivity of cells to stress conditions can vary among different strains of the same microorganism grown under the same conditions. The selection of particular strains and number of strains used to prepare the inoculum, as well as the number of cells in the inoculum, are, therefore, important considerations when designing

challenge tests for validation studies. Ideally, the physiological state of the test microorganism in a challenge inoculum should mimic that of cells that theoretically may be present in the test food. Otherwise, outcomes of challenge tests may have minimal value when attempting to validate conditions needed to control or inactivate microorganisms.

Development and Validation of Detection Methods – How Does the Physiology of the Target Cell Affect Assay Sensitivity?

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Detection of bacteria in foods and food environments represents a number of considerable challenges. For example, it is well known that bacterial cells exposed to certain stress conditions may enter an injured or a viable-nonculturable state, thus becoming undetectable on some or all media. In addition, bacteria in different growth phases or grown under different environmental conditions may differ considerable in (i) susceptibility to lysis procedures used in molecular detection assays, (ii) numbers of bacterial chromosomes present in a single cell, (iii) protein expression patterns, and (iv) mRNA transcript levels. All of these factors can have a considerable effect on the sensitivity of assays that (i) detect bacterial antigens (e.g., ELISAs and other antibody-based strategies), (ii) detect DNA (e.g., PCR assays), and (iii) detect RNA. While use of standardized enrichment procedures prior to detection provides some assurance that bacteria have been exposed to an at least somewhat standardized environment prior to detection, thus reducing variability in the physiology of bacterial cells that are used as inputs for detection assays some variability (e.g., with regard to growth phase) remains. Efforts towards direct detection of bacteria from foods or food-associated environments (i.e., without an enrichment step) though will yield bacterial cells that may differ tremendously in their physiology and gene expression patterns. The development of new assays and assay formats thus requires food microbiologists to pay increased attention to the effects of bacterial physiology on the sensitivity of different detection methods.

S12 Back to the Future: How Clinical Microbiology Findings Today Predict the Food Microbiology Headaches for Tomorrow

Food safety is an evolving science with an increasingly sophisticated toolbox of techniques for pathogen detection. This symposium aims to show the predictive side of food microbiology and how future needs for pathogen detection in foods can be anticipated from new findings in epidemiology and clinical medicine. This will be demonstrated on a continuum – from the past with established pathogens (STEC, Hepatitis A) to the present with an evolving pathogen (noroviruses, Hepatitis E) to the future with possible foodborne pathogens (Clostridium difficile) or diseases with a potentially infectious foodborne etiology (irritable bowel disease).

ILSI Symposium

E. coli O157:H7 and Other STEC: How We Come to Test Foods (and What Does This Tell Us)?

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Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens causing severe human infections. Although STEC may belong to a variety of serogroups, STEC O157 is a major cause of human disease and serogroups O26, O111, O103 and O145 are reported increasingly worldwide. These enterohemorrhagic *E. coli* (EHEC) cause attaching and effacing lesion (*eae*) and are characterized by a low infectious dose, requiring very sensitive methods for food testing. A standardized method based on immunomagnetic enrichment is available for STEC O157, while for serogroups other than O157 the issue is still under debate. A possible approach aims at the detection of any STEC in the food sample by testing enrichment cultures for toxin production, and/or presence of *stx* genes. The STEC strains then can be characterized. An advantage is the identification of STEC belonging to any serogroup. A drawback is that the presence of low-pathogenic STEC will complicate the detection of EHEC, likely present in lower concentration, requiring the examination of a large number of samples. An alternative strategy is directly targeted to EHEC. This approach is mainly based on the use of PCR to assess the presence of *stx, eae,* and serogroup-associated genes. It has the advantage of being very sensitive and allowing a direct discrimination between samples containing low pathogenic STEC (positive for *stx* only) and samples likely contaminated by EHEC. In this respect, the additional presence of *eae* and serogroup-related genes will predict the level of risk for human health associated with the food sample.

Noroviruses: From Unknown Etiology to Major Pathogen in Our Food: Role of Better Diagnostics

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Noroviruses (NoVs) are now recognized as the most frequent cause of outbreaks of gastroenteritis associated with foodborne exposure and are associated with more than 85% of all outbreaks of acute non-bacterial gastroenteritis. CDC studies have estimated that approximately 30–50% of foodborne outbreaks are attributable to norovirus, and approximately one in ten Americans will become ill with norovirus gastroenteritis every year. Persons of all ages are susceptible but the young, elderly and sick may suffer more severe consequences of illness, such as dehydration, requiring medical attention. In closed settings such as nursing homes, cruise ships, the military, norovirus often is transmitted person-to-person resulting in high attack rates and

large outbreaks. Food can be contaminated at the source (i.e., irrigation) or at point of service (e.g., infected foodhandler). Recent advances in the harmonization of norovirus sequence regions used for back tracing and genotyping allows to establishing a standardized norovirus surveillance network (e.g., CaliciNet). Among the >20 different norovirus genotypes, the majority of norovirus outbreaks are caused by the GII.4 genotype which was first recognized in the mid-1990s and has been shown to be associated with several pandemics, including that of the winter of 2006–2007. Genetic analyses suggest an epochal evolution for GII.4 viruses over the last twenty years with periods of stasis followed by rapid evolution of novel epidemic strains likely induced by herd immunity. Recent research shows that host genetics may play an important role in susceptibility to norovirus infection and that individuals who express certain ABH histoblood group antigens (HBGAs) on their mucosal surfaces are more susceptible to infection. These HBGA molecules serve as a (co)receptor for the virus on cells, and recent research has shown that these ligands may also be used in assays to concentrate norovirus from foods. Although no specific therapy is available for norovirus, recent developments, such as the discovery of murine norovirus that replicates in cell culture, allow us to assess the effectiveness of food sanitizers to protect us better against norovirus foodborne disease.

Is Inflammatory Bowel Disease an Infectious Disorder?

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Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronically remitting intestinal inflammatory conditions, affecting approximately 1.3 million individuals in North America. The current hypothesis for the development of IBD is that on the base of a genetic defect, patients with CD and UC display an increased susceptibility to an environmental agent(s). There is solid evidence for a genetic predisposition in both diseases, but only limited data on the environmental agent(s) inducing and maintaining intestinal inflammation. Consistently, bacteria and their products have been implicated to play a critical role in IBD, though identification of specific strains and their effector proteins has not occurred. This presentation will briefly review basic immunology and recently identified mutations associated with IBD and what possible role these molecules play for intestinal microbiology in patients. It will further discuss specific bacteria that have been implicated in etiology and/or reactivation of disease, including *Mycobacterium avium* subspecies *paratuberculosis*, Adherent Invasive *Escherichia coli*, *Clostridium difficile*, *Bacteroides fragilis*, and enterotoxins B producing staphylococci. In addition, this summary will explore dysbiosis as seen in IBD and provide a brief outlook about the challenges for the future.

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

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

T1-01 DSC Expression of the Urease Operon in *Escherichia coli* O157:H7 Treated with 0.5% Sodium Benzoate

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Introduction: Sodium benzoate is a widely used food antimicrobial in drinks and fruit juices. Understanding gene regulation in *E. coli* O157:H7 under antimicrobial stress is critical to enable the design of appropriate control strategies. High-throughput novel technologies such as microarrays enable the study and characterization of gene expression.

Purpose: Microarrays were used to determine the transcriptional response of *E. coli* O157:H7 after exposure to 0.5% sodium benzoate.

Methods: Stationary phase *E. coli* O157:H7 grown in Luria-Bertani broth (LB) was centrifuged at 4°C, and the pellet was resuspended in 150 ml LB containing 0 (control) or 0.5% (w/v) sodium benzoate. Each treatment was duplicated, sampled at 0 (immediately after exposure), 5, 15, 30, and 60 min. RNA was extracted using the Qiagen RNeasy extraction kit and analyzed with the GeneChip *E. coli* 2.0 Array (Affymetrix). Intensity signals for each probe set were normalized with a GCRMA algorithm. Significantly regulated genes (positive-false discovery rate < 0.05) were determined using the R-MAANOVA (microarray analysis of variance) package.

Results: Microarray analysis revealed that all genes in the urease operon were up-regulated >2-fold (linear-scale) after 60 min exposure to sodium benzoate. The urease operon is composed of three structural genes, ureA, ureB, and ureC, and four accessory genes, ureD, ureE, ureF, and ureG. Validation of expression of the structural genes by real-time-RT-PCR is ongoing.

Significance: Urease catalyzes the hydrolysis of urea to ammonia and carbon dioxide, and is one mechanism by which microorganisms survive in acidic environments. Ammonium ions accumulate in the surrounding environment, effectively mediating a drop in pH. In this study, exposure of *E. coli* O157:H7 to sodium benzoate in a neutral pH environment showed increase in transcription of the entire urease operon. Understanding adaptations of *E. coli* O157:H7 after exposure to antimicrobials is essential to better understand and implement methods to inhibit or control its survival in foods.

T1-02 Polylysine-Induced Sensitization of *Enterobacteriaceae* to Medium-Chain Fatty Acid Derivatives

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Introduction: The outer membrane (OM) of Gram negative bacteria is an effective permeability barrier against the inhibitory action of medium chain fatty acid derivatives. This suggests that Gram negatives such as *Salmonella* and *E. coli* can be sensitized to these derivatives by disorganization of the OM. Epsilon-polylysine (ε-polylysine) is a membrane disorganizing agent.

Purpose: The purpose of this study was to test the growth inhibition of *Salmonella* and *E. coli* O157:H7 by combinations of ε-polylysine and monoglycerides or lactic acid esters of medium chain fatty acid esters (lactylates).

Methods: A [10 × 10] "checkerboard" procedure was followed to study the combined effects of ε-polylysine and a fatty acid derivative in broth. This allows varying the concentrations of the antimicrobials and results in 100 different combinations. Growth rate of the cultures was determined with a Bioscreen C, which is a precision incubator capable of simultaneously measuring the growth of up to 200 cultures by vertical spectrophotometry. Interactions of the antimicrobials were evaluated according to the Bliss criterion for independence and the Loewe criterion for dose additivity. Finally the ability of lactylates and monoglycerides alone and in combination with ε-polylysine to inactivate *Salmonella* and *E. coli* O157:H7 on raw ground beef was studied.

Results: Growth of *Salmonella* or *E. coli* O157:H7 in broth was unaffected by < 0.04% ε-Polylysine, < 0.25% caprylic (C8)-lactylate or < 0.1 % caprylic (C8) monoglyceride. Inhibition of both organisms was nearly complete in media containing a combination of ε-polylysine (0.01–0.04%) and caprylic (C8)-lactylate (0.05–0.25%) or a combination of ε-polylysine (0.01–0.04%) and caprylic (C8)-monoglyceride (0.02–0.1%). Similar observations were made with combinations of ε-polylysine with C10-, C12- or C14-lactylates or monoglycerides. Synergy between ε-polylysine and the fatty acid derivatives was also observed in meat systems although higher concentrations of ε-polylysine and fatty acid derivatives were required to achieve an effect similar to that observed in broth systems.

Significance: This study highlights the potential of combinations of ε-polylysine and certain monoglycerol- and lactic acid esters of C8 – C14 fatty acids to suppress the growth of *Salmonella* and *E. coli.*

T1-03 Gene Expression Profiling of *Listeria monocytogenes* Strain F2365 in UHT Pasteurized Skim Milk

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Introduction: Listeria monocytogenes is a foodborne pathogen of significant threat to public health. *L. monocytogenes* has the ability to grow or survive at refrigeration temperatures and under conditions of relatively low pH, high salt and low water activity in foods. However, the factors contributing to the survival and growth of such strains in food remain unclear.

Purpose: Monitor the gene expression profiles of *L. monocytogenes* strain F2365 in UHT pasteurized skim milk, using microarray technology.

Methods: Total RNA was isolated from strain F2365 in UHT pasteurized skim milk after 24 h at 4°C, labeled with fluorescent dyes, and hybridized to custom-made oligonucleotide (35-mers) microarray chips containing the whole genome of the serotype 4b strain F2365 of *L. monocytogenes.*

Results: Compared to *L. monocytogenes* grown in brain heart infusion broth (BHI) for 24 hours at 4°C, 26 genes were up-regulated (> 2-fold) in UHT pasteurized skim milk, whereas 14 genes were down-regulated

(< -2-fold). The up-regulated genes included genes encoding for transport and binding, transcriptional regulators, proteins in amino acid biosynthesis and energy metabolism, protein synthesis, toxin production and resistance, cell division, and hypothetical proteins. The down-regulated genes included genes that encode for transport and binding, protein synthesis, cellular processes, cell envelope, energy metabolism, and a transcriptional regulator and an unknown protein. The gene expression changes determined by microarray assays were confirmed by real-time RT-PCR analyses. Furthermore, cells of *L. monocytogenes* in skim milk displayed the same sensitivity to oxidative stress as cells grown in BHI, demonstrating that the elevated levels of genes encoding for manganese transporter complexes in milk did not result in changes in oxidative stress sensitivity.

Significance: This study represents the first report on global transcriptional gene expression profiling of *L. monocytogenes* in a liquid food.

T1-04 DSC Interactions between σ^B and σ^L Appear to Contribute to *Listeria monocytogenes* Antimicrobial Resistance

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Introduction: Resistance of the foodborne pathogen *Listeria monocytogenes* to antimicrobials is of significant concern to public health and the food industry. To evaluate factors contributing to *L. monocytogenes* antimicrobial resistance, the putative bacteriocin immunity gene *lmo2570* (predicted to be σ^B-dependent), σ^B and σ^L were assessed for their contributions to antimicrobial resistance.

Purpose: The aim was to determine if *lmo2570*, σ^B and/or σ^L contribute to antimicrobial peptide resistance in *L. monocytogenes.*

Methods: We performed qRT-PCR on mRNA from *L. monocytogenes* 10403S, Δ*sigB*, and Δ*sigL* strains grown to logarithmic phase and exposed to sub-minimal inhibitory concentration of 75 AU/ml nisin for 10 min. Spot-on-lawn assays were conducted to study the growth of *L. monocytogenes* in the presence of various bacteriocin-producing *B. subtilis* strains. Lastly, to evaluate contributions of *lmo2570*, σ^B and σ^L, the 10403S, Δ*sigB*, and Δ*sigL* strains were grown in BHI broth and exposed to 150 AU/ml nisin for 9 hours.

Results: TaqMan qRT-PCR confirmed *lmo2570* is σ^B-dependent. However, *lmo2570* transcript levels were not affected by nisin in 10403S, Δ*sigB* or Δ*sigL* strains. Spot-on-lawn assays demonstrated that while the Δ*lmo2570* and Δ*sigL* strains were not inhibited by *B. subtilis*, Δ*sigB*/Δ*sigL* was inhibited by two *B. subtilis* strains and Δ*sigB* was inhibited by one strain as compared to growth of the10403S strain ($P < 0.05$). Based on initial analysis of four replicates, consistent trends of reduced survival or initial increased survival of the Δ*sigB*/Δ*sigL* and the Δ*sigB* strains, respectively, occurred in the presence of nisin, as compared to 10403S ($P < 0.05$).

Significance: While *lmo2570* is not essential for *L. monocytogenes* 10403S growth in the presence of bacteriocins, σ^B, in conjunction with σ^L, is important for antimicrobial resistance. Specifically, inhibited growth of the Δ*sigB*/Δ*sigL* and the Δ*sigB* strains in the presence of some *B. subtilis* strains or nisin suggests a role for interactions between σ^B and σ^L in antibiosis regulation.

T1-05 Influence of Oxygen on Survival and Quantification of *Escherichia coli* O157:H7 and *Salmonella* Serovar Typhimurium in Manure and Slurry

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Introduction: The survival and spread of *E. coli* O157:H7 and *Salmonella* serovar Typhimurium is greatly affected by the way manure is stored and applied. The presence or absence of oxygen in manure and slurry may lead to differences in prevalence and survival of the pathogens. Moreover, the quantification of these facultative anaerobic pathogens on Petri plates with surveys or survival experiments may be affected by the presence or absence of oxygen.

Purpose: Various experiments were conducted to investigate the survival of *E. coli* O157:H7 and *Salmonella* serovar Typhimurium in aerobic and anaerobic manure and slurry as a function of plating procedure (aerobic vs. anaerobic).

Methods: Fresh samples of manure and slurry were collected and analyzed for chemical and biological parameters. *E. coli* O157:H7 and *Salmonella* serovar Typhimurium were introduced into the substrates at 10^7 log CFU/g and stored under aerobic and anaerobic conditions. Survival was assessed with selective plating, whereby the plates were incubated aerobically and anaerobically. The log-transformed data over time were fitted to the Weibull survival model. The parameters of the Weibull model were analysed statistically (ANOVA) for differences between the treatments.

Results: The estimated survival times showed that *E. coli* O157:H7 survived significantly longer under anaerobic conditions (181 days versus 13 days, $P < 0.001$). On average, in 56.3% of the samplings, the number of recovered *E. coli* O157:H7 cells by anaerobic incubation of Petri plates was significantly ($P < 0.05$) higher in comparison with standard procedure. Survival of *Salmonella* serovar Typhimurium was not different between aerobic and anaerobic storage of manure or slurry, nor was it different between aerobic and anaerobic incubation of Petri dishes.

Significance: The results indicate that care should be taken with storing manure under anaerobic conditions. The difference in decline of *E. coli* O157:H7 illustrates the important role of (autochtonous) microbial community and chemical processes. *Salmonella* serovar Typhimurium was shown to be the less sensitive to different environmental stresses. Moreover, anaerobic incubation of Petri dishes may improve the sensitivity of quantitative sampling procedures, especially for enumeration of *E. coli* O157:H7.

T1-06 DSC Survival of Norovirus in Biosolids

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Introduction: Noroviruses cause over 90% of non-bacterial epidemic gastroenteritis worldwide and epidemiological projects in 1999 indicated that noroviruses may account for over 60% of all foodborne disease in the US. Norovirus is shed in the feces of infected humans and animals and can contaminate fresh produce by contamination of the irrigation waters or through contaminated biosolids and manure.

Purpose: The objective of this study is to understand factors controlling the survival of viruses in agricultural systems.

Methods: Murine Norovirus Virus 1 (MNV-1, ~ $2*10^5$ PFU/ml) was added into two different types of biosolids, pellet treated (PT) and Alum treated (AT). Extraction of MNV from biosolids with 3% beef extract or Phosphate buffer (Na_2PO_4, pH 9.5) was not possible, even for sample stored at time 0. Instead, 10 ml Phosphate buffer was added to 2 g biosolids, and viral RNA was directly extracted from the solution using Qiagen Viral RNA Extraction Kit (Qiagen, CA). The standard curve of Real-time PCR was generated by serial dilution of biosolids solution, RNA extraction and two-step Real-time PCR with Qiagen SYBR Green Kit (Qiagen, CA). The Most Probable Number (MPN) method was used to detect the infectivity of MNV in biosolids.

Results: The MNV-1 contaminated biosolids (PT and AT) were incubated at 20 or 4°C up to 60 days and analyzed every 10 days. After 60 days incubation, Real-time PCR showed a ~5 log reduction of MNV in PT at 20°C and ~3 log at 4°C; for MNV in AT, there was a ~2 log reduction at 20°C and 1 log at 4°C. The MPN showed that there were ~3 and 4 log PFU/ml MNV still infectious after 60 days in AT at 20 or 4°C.

Significance: The survival of MNV-1 is dependent upon biosolid type, treatment, and storage conditions.

T1-07 DSC Effect of Zero-Valent Iron on Removal of *Escherichia coli* O157:H7 from Agricultural Waters

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Introduction: A novel water filtration system using zero-valent iron (ZVI) is being investigated as a simple and inexpensive approach to reducing *E. coli* O157:H7 in water for both pre-and post-harvest processes.

Purpose: This study was initiated to determine the effectiveness of zero-valent iron in the removal and inactivation of *E. coli* O157:H7 in a simulated irrigation system.

Methods: The 2006 spinach and lettuce outbreak strains of *E. coli* O157:H7 (10^6 CFU/ml in 1600 ml artificial groundwater (AGW) of pH 5, 7.5 and 9) were pumped through sand (control) and iron columns with a flow rate of 1 ml/min at 4°C. An initial pulse of 10 pore volumes (PV) was pumped through the columns, followed by a flush of sterile AGW (20 PV). Effluent samples were collected at 4.5 and 9 minute intervals for the pulse and flush. One-ml samples from each tube were serially diluted in buffered peptone water and plated onto TSA with 50 µg/ml nalidixic acid in duplicate; bacterial concentrations were determined after 24 hours at 37°C. Following the experiments, the columns were dismantled and examined for the presence of *E. coli*.

Results: Break-through curves at all pH values showed reduction of 1–2 logs of *E. coli* O157:H7 by the inclusion of zero-valent iron compared to sand. During the pulse, removal efficiencies by ZVI were 1.78 ± 0.11, 1.01 ± 0.19, and 1.5 ± 0.23 logs for pH 5, 7.5, and 9. Removal remained constant throughout the pulse. Bacterial inactivation by ZVI was observed to be 2.38 ± 0.67 logs as compared to sand column at identical locations.

Significance: The ability of ZVI to remove and inactivate *E. coli* O157:H7 shows great potential for reducing irrigation water contamination. ZVI is currently being assessed for its effectiveness on wash water for leafy greens and the filtration system is being optimized for enhanced removal.

T1-08 DSC Transcriptome Analysis of *Escherichia coli* O157:H7 under Acidic Conditions

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Introduction: Escherichia coli O157:H7 can survive under acidic environments (pH < 3.0) and grow under mildly acidic conditions (pH 4.5–6.0). Gene expression analysis of *E. coli* O157:H7 under acid stress is critical to the design of control strategies to decrease foodborne outbreaks. Functional genomics using microarrays and validation by RT-PCR are novel tools that enable gene expression analysis.

Purpose: Microarrays were used to analyze gene expression of *E. coli* O157:H7 under acidic conditions similar to those associated with apple cider processing.

Methods: E. coli O157:H7 was grown to 7 log CFU/ml in LB broth as follows: control (pH 7; 37°C); acid shocked (pH 7; 6 h at 37°C, then pH reduced to 3.5 for up to 60 min); and acid adapted (pH 5; 12 h at 37°C, then pH reduced to 3.5 for up to 60 min). RNA was extracted using the Qiagen RNeasy extraction kit and analyzed using the GeneChip *E. coli* 2.0 Array (Affymetrix). Microarray results were analyzed using GC-RMA, ANOVA and *t*-test to compare treatments, with significance at $P < 0.01$ and a cut-off of 2-fold difference (linear scale) in gene expression, and validated by real-time RT-PCR.

Results: Microarray analysis of acid shocked cells revealed up-regulation of genes related to iron (cyo, ent, fhu, fep, and feo operons); genes related to electron transport and metabolism were up- or down-regulated ($P < 0.01$). Genes for DNA binding, biosynthesis, iron, transport, and cellular metabolism were up-or down-regulated ($P < 0.01$) in acid adapted cells, with expression of the cyclopropane fatty acid biosynthesis (cfa) gene and glutamate decarboxylase acid resistant systems significantly up-regulated. Virulence genes (eae, *stx*1, or *stx*2) were not significantly differentially expressed.

Significance: Data from microarray analysis suggest that acid adapted and acid shocked *E. coli* O157:H7 utilize different genes for survival. Common to both are iron regulation and transport, which can be potential targets in control strategies.

T1-09 DSC Transcriptional Regulators SigB and PrfA, and Flagella, Interact in a Temperature-Dependent Manner to Facilitate *Listeria monocytogenes* Invasion

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Introduction: Listeria monocytogenes SigB and PrfA regulate the transcription of a number of virulence genes. Even though PrfA activity and expression of flagella, which have been shown to contribute to invasion, are temperature dependent, flagella expression is PrfA independent. This suggests multiple mechanisms of temperature dependent regulation of virulence genes.

Purpose: The purpose of this study was to evaluate the effect of growth temperature on (i) the SigB and PrfA-dependent invasion gene transcription and invasion phenotypes and (ii) flagella transcription and phenotypic expression, including temperature dependent interactions.

Methods: L. monocytogenes 10403S, Δ*sigB*, Δ*prfA*, Δ*sigBprfA*, Δ*flaA*, and Δ*inlAflaA* grown to early stationary phase at 30°C or 37°C were used for Caco-2 invasion assays at 37°C. Using qRT-PCR, transcript

levels of *inlA*, *flaA*, *plcA* (indicator of PrfA activity) and *gadA* (indicator of SigB activity) were determined for 10403S, Δ*sigB*, Δ*prfA*, and Δ*sigBprfA* grown at 30°C or 37°C; in addition, *prfA* and *sigB* transcript levels were determined for 10403S.

Results: For bacteria grown at 37°C, deletions of *sigB*, *prfA*, and *flaA* (as well as the *sigB*prfA* interaction) significantly affected Caco-2 invasion ($P \leq 0.05$); at 30°C only *sigB* and *flaA* deletions significantly affected invasion. At both temperatures, an interaction between the *inlA* and *flaA* deletions had an effect on invasion. Only *flaA* showed temperature dependent transcript levels (i.e., higher levels at 30°C) with apparently negative regulation of *flaA* transcript levels by SigB only at 37°C ($P = <.0001$). *plcA* transcript levels were PrfA-dependent at 37°C, but not at 30°C ($P < 0.05$), while *inlA* transcript levels were SigB-dependent at both temperatures.

Significance: Our data suggest that *L. monocytogenes* invasion may depend on a complex interaction of transcriptional regulators and flagella, and that this interaction is temperature dependent. Thus, *L. monocytogenes* grown under different temperatures and conditions may differ in their ability to invade human cells.

T1-10 Virulence Attenuated *Listeria monocytogenes* Strains Commonly Isolated from Food Show Potential to Confer Protective Immunity

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Introduction: Listeria monocytogenes may cause severe systemic infections in high-risk individuals and inlA plays an important role in this pathogen's ability to invade host cells. More than 30% of *L. monocytogenes* isolates from food carry mutations leading to a premature stop codon (PMSC) in inlA, resulting in production of a truncated and secreted Internalin (InlA).

Purpose: This study was conducted to probe the causal relationship between inlA PMSC mutations and virulence and to assess the potential for *L. monocytogenes* strains carrying a PMSC in inlA to function as natural vaccines.

Methods: Guinea pigs were intragastrically challenged with paired isogenic *L. monocytogenes* strains, which share the same genetic background and only differ by the presence or absence of an inlA PMSC, to determine the role of these mutations in virulence. We evaluated the ability of virulence-attenuated *L. monocytogenes* strains carrying an inlA PMSC to confer protective immunity by first infecting guinea pigs with a virulence-attenuated *L. monocytogenes* strain (vaccination) followed by infection with a fully-virulent *L. monocytogenes* strain (challenge) 15 d post-vaccination. Animals were euthanized at 72 h post-challenge and *L. monocytogenes* populations from the liver, ileum, mesenteric lymph nodes, and spleen were enumerated.

Results: L. monocytogenes populations recovered from animals infected with strains carrying PMSC mutations in inlA were significantly ($P = 0.005$) lower overall than counts recovered from animals infected with paired isogenic strains encoding a full-length InlA. Significantly lower ($P < 0.05$) *L. monocytogenes* populations were recovered from the liver, ileum, mesenteric lymph nodes, and spleen of vaccinated animals compared to unvaccinated controls challenged with either a laboratory control strain or serotype 4b outbreak strain.

Significance: Our findings support that naturally occurring virulence-attenuated *L. monocytogenes* strains may play a role in maintaining population immunity because of their common presence in food and ability to confer protection against infection by fully virulent strains.

T1-11 DSC Characterization of the Ability of Bovine *Escherichia coli* O157 to Adhere to Human Intestinal Epithelium Cells

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Introduction: The ability of *Escherichia coli* O157 to cause gastrointestinal or renal complications, such as hemorrhagic colitis and hemolytic uremic syndrome, depends on the organism's ability to attach to intestinal epithelium and produce shiga-like toxins.

Purpose: This study was conducted to assess the pathogenic potential of different *E. coli* O157 genotypes.

Methods: E. coli O157 isolates collected over six samplings from a cattle population were characterized by (i) multiplex PCR to detect *E. coli* O157:H7 specific sequences (rfbE and fliCH7) along with virulence factors (eaeA, *stx*I and *stx*II) and (ii) pulsed-field gel electrophoresis (PFGE). The pathogenic potential of *E. coli* O157 isolates representing different genotypes was assessed by their ability to adhere to Caco-2 human intestinal epithelial cells.

Results: E. coli O157:H7 isolates lacking either *stx*I or *stx*II demonstrated the greatest ($P < 0.05$) ability to attach to Caco-2 cells. PFGE typing of isolates revealed a dominant subtype representing > 50% of isolates characterized. This dominant subtype persisted within the cattle population as indicated by isolation of this subtype at a frequency ranging between 32–88% over six collections. *E. coli* O157:H7 isolates belonging to the dominant subtype demonstrated greater ($P < 0.001$) attachment efficiency for Caco-2 cells compared to isolates differing by three or more bands. There was a clear correlation ($P < 0.001$) between genetic divergence from the dominant subtype and decreased attachment efficacy, where isolates differing from the dominant subtype by seven bands showed the lowest attachment efficiency.

Significance: Our data show that certain *E. coli* O157:H7 strains may persist in cattle populations, which may be explained by an enhanced ability to adhere to the intestinal epithelium. *E. coli* O157:H7 strains that persist in cattle are more likely to be transmitted to humans and may demonstrate greater virulence, due to an enhanced ability to attach to human intestinal epithelial cells.

T1-12 DSC Molecular Epidemiology and Characterization of Virulence Gene in *Yersinia enterocolitica* Isolated from Swine

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Introduction: Yersinia enterocolitica is an important foodborne pathogen known to cause gastrointestinal problems with symptoms ranging from acute enteritis with fever to occasionally bloody watery diarrhea, particularly in children (Bottone, 1997). It is estimated to

cause 96,000 cases of human disease annually in the United States (Mead et al., 1999). Swine are recognized as a major reservoir and potential source of pathogenic *Y. enterocolitica* to humans.

Purpose: The goal of the study was to characterize virulence determinants from *Y. enterocolitica* isolated from swine and determine the occurrence of strains that are of pathogenic significance to humans.

Methods: A total of 172 *Y. enterocolitica* isolated from swine farms and slaughter plants from two production types (antimicrobial free and conventional) in three geographic locations (North Carolina, Ohio and Wisconsin) were studied for their phenotypic and genotypic diversity. The isolates were tested for their antimicrobial susceptibility, using Sensititre microdilution panels, and serotyped with O antisera. Polymerase chain reaction was performed to test for the presences of chromosomal virulence genes, namely ail, ystA and ystB and plasmid virulence gene yadA. Genotyping using amplified fragment length polymorphism (AFLP) was performed to study the clonality or diversity of the strains.

Results: A total of 12 antimicrobial resistance patterns were observed and the predominant pattern was pan-susceptible (67.4%), followed by ampicillin (19.8%) and sulfamethoxazole (2.9%). The common serotype was O:3 (37.8%) followed by O:5 (29.1%) and O:9 (4.7%). Forty-four were untypable. Sixty-two (36%) strains were found to carry the ail gene. The ystA and ystB gene were detected in 83.1% and 26.2% of the strains, respectively. None was positive for the plasmid virulence gene yadA. In 18 of the isolates, all three virulence genes were present. AFLP fingerprinting revealed 19 clusters and a higher percentage of the isolates that were grouped in one cluster originated from the same geographic locations and or production system.

Significance: This study indicates that swine do carry pathogenic *Y. enterocolitica* strains and can be an important source of human infection.

T2-01 DSC Mechanisms of Allyl Isothiocyanate Antimicrobial Activity against *Escherichia coli* O157:H7

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Introduction: Allyl isothiocyanate (AIT) from cruciferous plants has a broad spectrum of bactericidal activity, including against *E. coli* O157:H7. AIT is a strong electrophile, and it is likely that its antimicrobial activity results from reaction with a variety of substances, including hydroxyls, amines and thiols. Interference with enzymatic activity has been proposed as a mechanism of AIT inhibitory action, but little work has been done and interpretation is complicated by the instability of AIT in water.

Purpose: Evaluate the importance of AIT degradation products upon its antibacterial activity against *E. coli* O157:H7 and analyze AIT interaction with thioredoxin reductase, a thiol-containing enzyme involved in ribonucleotide synthesis.

Methods: HPLC was used to characterize breakdown products from AIT in an aqueous solution. AIT was recovered using hexane and the aqueous phase containing hydrophilic AIT breakdown products was tested against a cocktail of 5 strains of *E. coli* O157:H7. The main component in the aqueous phase was characterized by HPLC and LC-MS and also tested for antibacterial activity. AIT interaction with *E. coli* thioredoxin reductase activity was evaluated in vitro using a colorimetric assay. Growth and enzymatic inhibition were monitorated and compared, using ANOVA.

Results: After 24 h in water, AIT was partially decomposed into three different products, which together did not inhibit bacterial growth. However, they showed significant antibacterial activity ($P < 0.01$) when combined with a sub-lethal dose of AIT (10 ppm). The main breakdown product, diallylthiourea (~90%), showed similar results in the presence of 10 ppm AIT. Moreover, AIT alone was able to inhibit thioredoxin reductase at doses as low as 1 ppm ($P < 0.05$).

Significance: These results suggest that both AIT and its degradation products are likely involved in the antibacterial action of AIT via inhibition of essential metabolic activity of microbial cells.

T2-02 DSC Antimicrobial Effects of Persimmon Puree Concentrate in Brain Heart Infusion on *Listeria monocytogenes* and *Escherichia coli* O157

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Introduction: Repeated pathogen-related recalls in the food industry have increased the need for methods to safely and naturally reduce microbial contamination. Persimmons have been known to contain astringent and bitter compounds, such as phenolics, which break down into gallic and ellagic acid and suppress microbial populations. Persimmon puree can be a natural alternative that could be incorporated into food products to naturally control pathogens.

Purpose: The purpose of this experiment was to evaluate the efficacy of persimmon puree in Brain Heart Infusion (BHI) to suppress microbial growth at various concentrations.

Methods: Persimmon puree (La Vigne Organics, California) was added at 0, 1, 3, 5 and 10% to sterile BHI. Stomacher bags were then inoculated with BioBall™ 30 ± 2 CFU of *Escherichia coli* O157 (Ec O157) and BioBall™ 30 ± 2 CFU *Listeria monocytogenes* (Lm) individually at each concentration. Samples were taken at 0, 6, 12, 24, 36, and 72 hours and plated in duplicate onto MacConkey Sorbitol Agar (MSA) and Modified Oxford Medium Agar (MOX) for three replications of each bacterium.

Results: Samples from the defrosted original puree showed 1.80 log CFU/g total aerobic count. Because of background Gram (-) microflora, inhibition of Ec O157 on MSA could not be ascertained. Persimmon puree was not found to be effective in inhibiting Gram (-) bacteria at any concentration at 37°C in 24 h. However, Lm results showed there were significant differences between microbial growth in BHI control vs. 1, 3, 5, and 10% puree, with 3.13, 5.65, 5.85, and 6.19 log CFU/ml, respectively, suppression of growth of Lm at 37°C in 24 h.

Significance: These data suggest that persimmon puree could be an effective antimicrobial against Lm bacteria in a liquid system. The effectiveness on Ec O157 and other Gram (-) bacteria is being researched further.

T2-03 DSC Feedlot Production Practices and Their Impact on Pre- and Post-Harvest Antimicrobial Susceptibility Patterns of *Enterococcus* spp.

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Introduction: Antimicrobial-resistant enterococci are of great concern in human health, especially in nosocomial infections. Many argue that agriculture's use of antimicrobials is a contributing factor to the increasing burden of resistance in some pathogens. However, only a limited amount of data exist on the impact of animal production practices on the emergence of resistance.

Purpose: To evaluate the effects of subtherapeutic administration of antimicrobial drugs on phenotypic characteristics of *Enterococcus* spp.

Methods: Treatments were randomly assigned to 1600 feedlot steers, including 1) monensin (29.8 mg/kg DM) and tylosin (9.7 mg/kg DM), 2) tylosin only, 3) monensin only, and 4) no antimicrobials added. Fecal samples were collected at arrival, re-implant, and prior to shipment, while hide and carcass samples were collected at harvest. Enterococci concentration, species, and minimum inhibitory concentration of antimicrobials were estimated using standard microbiological techniques.

Results: 88.7% of isolates were resistant to at least one antimicrobial. The mean number of drugs to which isolates were resistant was 3.6, 3.5, 4.1, and 4.0 in control, rumensin, tylosin, and combination cohorts respectively, with no significant differences among treatments. 33.8% (n = 2137) of isolates presented MLS resistance phenotype. No 3-way interaction was detected in fecal or surface samples for tylosin or lincomycin ($P > 0.10$). An interaction between tylosin and period was seen for both tylosin and lincomycin in fecal samples ($P < 0.0001$) where exposed isolates at reimplant and harvest had greater MIC's than unexposed.

Significance: Exposure to tylosin and rumensin had a negligible effect on antimicrobial resistance development. The majority of species recovered were unimportant to human health. Potential human contamination of carcasses could explain increase in *E. faecalis* presence on carcasses; however, in-plant interventions seem effective in elimination of potential pathogens.

T2-04 Effective Reduction of *Escherichia coli* O157:H7 in Feedlot Cattle by Use of Bacteriophages

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Introduction: Escherichia coli O157:H7 is a pathogen causing bloody diarrhea and hemolytic uremic syndrome in infected humans, but is a normal resident of the gut of cattle. Consumption of meat contaminated with this pathogen is a major cause of human infections. The industry needs an effective method to reduce *E. coli* O157:H7 in beef cattle, and consequently in beef processing, which would have a major impact on reduction of infections in humans. Its implementation would also help the beef processing industry by reducing financial losses due to recalls, plant production slowdowns and related losses. Reducing the pathogen load in animals before they go to slaughter will help address all these issues.

Purpose: Develop an all-natural technology that will reduce *E. coli* O157:H7 load in feedlot cattle without disrupting current feedlot practices.

Methods: Bacteriophages that specifically infect and kill *E. coli* O157:H7 were isolated from the feedlot environment, and characterized in detail, and their safety confirmed by molecular techniques. Formulations best suited for effective delivery in cattle were developed, and administered in feed to naturally-infected cattle (80 animals) with standard feedlot practices, and the reduction in *E. coli* O157:H7 was monitored.

Results: A large library of phages effective against multiple field isolates of *E. coli* O157:H7 has been established, suitable for use as active pharmaceutical ingredients in a formulated product. A three-phage product was developed. This product was shown to reduce *E. coli* O157:H7 shedding in naturally contaminated cattle by up to 99%. No bacterial isolates resistant to our phage product were observed.

Significance: We have developed an all-natural non-antibiotic product for the reduction of *E. coli* O157:H7 in cattle prior to slaughter, reducing contamination of meat. This treatment will have an impact on the beef processing industry at several levels: reducing their monetary losses due to contaminated meat recalls and other processing-related losses, reducing legal liability and increasing product acceptability by the consumer.

T2-05 Efficacy of Four Antimicrobial Ingredients to Inhibit the Growth of *Listeria monocytogenes* in Roast Beef

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Introduction: Listeria monocytogenes is a Gram-positive microorganism, ubiquitous in the environment and often found on Ready-to-eat meats. *L. monocytogenes* has been at the forefront of concern in the food industry for the past few years. This microorganism poses a critical threat due to its widespread abundance in the environment, its ability to grow at a wide range of temperatures, and the severe illness it causes when consumed.

Purpose: The purpose of this study was to compare the inhibition of growth of *L. monocytogenes* on roast beef by the use of pioneering antimicrobials.

Methods: Patented, proprietary blends of vinegar and lemon juice concentrate (MOstatin LV1), vinegar (MOstatin V), buffered sodium citrate and sodium diacetate (IONAL LC), and potassium lactate and vinegar (Opti.form Vinegar), along with control samples containing no antimicrobials, were evaluated in roast beef for the ability of the blends to limit the growth of *L. monocytogenes* and extend shelf life. Each sample was surface inoculated with a four-strain cocktail of *L. monocytogenes* at 3–4 log CFU/g, vacuum packaged and stored at 4°C for 120 days. The samples were analyzed for cook yield, brine pH, aerobic plate counts, and *L. monocytogenes* by plating on Tryptic Soy Agar with a MOX overlay.

Results: From day 1 to 120 MOstatin LV1, MOstatin V, and IONAL LC demonstrated < 0.5 log CFU/g growth of *L. monocytogenes*. Opti.form Vinegar exhibited > 2 log CFU/g growth of *L. monocytogenes* by day 112. There were differences ($P < 0.05$) in APC values between treatments throughout the study.

Significance: These data suggest that blends of vinegar and lemon juice concentrate (MOstatin LV1), vinegar (MOstatin V), and buffered sodium citrate and sodium diacetate (IONAL LC) have a highly inhibitory effect on the growth of *L. monocytogenes* on roast beef.

T2-06 *Listeria* Control in Ready-to-Eat Meat by Use of Clean Label Ingredients

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Introduction: Listeria control in Ready-to-Eat (RTE) meats, especially uncured products, still creates a challenge for meat and poultry processors. Existing ingredient technologies can have a negative flavor impact when used at levels sufficient to control *Listeria*. Additionally, retailers and consumers are demanding clean label ingredients in food products. PURAC has developed a new clean label product, VERDAD 55, which shows superior *Listeria* control in uncured meat and poultry products compared with lactate/diacetate technology.

Purpose: To demonstrate control of *Listeria monocytogenes* in uncured turkey through 90 days storage with VERDAD 55, a clean label ingredient.

Methods: A high moisture, uncured turkey product was manufactured with a brine containing sodium chloride, sodium triphosphate, maltodextrin, carrageenan, starch and VERDAD 55. The cooked turkey rolls were inoculated with a suspension of four *Listeria monocytogenes* strains to a final level of about 1000 CFU per g product and stored at 4°C. The product was sampled in duplicate through 90 days of storage. A suitable dilution of the sample was plated on Palcam CM0877 agar fortified with Palcam selective supplement SR0150.

Results: The outgrowth of *Listeria monocytogenes* in the control turkey rolls reaches 2 log growth in 7 days. In comparison Turkey containing 2.5% Opti.Form PD4 and 3.5% VERDAD 55 reaches 2 log growth at 28 and 80 days, respectively. Turkey containing 4.5% VERDAD 55 shows no growth of *Listeria* through the 90 day testing period at 4°C.

Significance: VERDAD 55 provides a clean label solution that enables processors to control *Listeria* growth in uncured turkey products through 90 days of storage, and therefore enables processors to gain Alternative 2 status on these high-risk items.

T2-07 Bacteriophage Treatment Reduces *Campylobacter jejuni* in Clinically Infected Chicken

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Introduction: Campylobacter jejuni is an important etiological agent causing gastroenteritis in humans and has been linked to the poultry industry. Contamination of chicken by this pathogen is a major food safety issue in this sector, causing significant monetary losses to the processor. Producers and processors are looking for novel methods to reduce levels of this pathogen in poultry meat. There is also regulatory pressure for such reductions, both in North America and in the EU.

Purpose: Develop a non-antibiotic based treatment to reduce *Campylobacter jejuni* contamination in poultry

Methods: A bacteriophage library containing phages having a broad host range has been developed and selected phages formulated for administration in poultry feed. Broiler chicken were infected with a field isolate of *Campylobacter jejuni* at 3 weeks of age and a week later treated for 5 days with a preparation containing two *Campylobacter* phages. A total of 50 birds (25 control and 25 treated) were used in this study. Caecal colonization of *Campylobacter* was monitored in the birds through the treatment period

Results: Bacteriophages were effectively delivered to chicken by administering in feed. Treatment with our bacteriophage preparation reduced *Campylobacter jejuni* counts in the caeca of phage treated birds by about two logs by the end of the treatment period as compared to the control group. No adverse effects were observed in chicken upon treatment with our phage product.

Significance: We have developed a non-antibiotic on-farm approach for reducing *Campylobacter jejuni* contamination of poultry. This treatment method will help the poultry producer to reduce *Campylobacter jejuni* contamination of the entire flock of birds before sending them to slaughter, thus reducing overall load of this pathogen in the production/processing chain.

T2-08 Antimicrobial Resistance in *Campylobacter* Isolates Recovered from Chicken Carcass Rinsates in 2007

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Introduction: Since the 1990s there has been increasing concern regarding the development of antimicrobial resistance among foodborne bacteria. To monitor resistance trends in foodborne bacteria, the United States National Antimicrobial Resistance Monitoring System (NARMS) tracks antimicrobial susceptibility among *Campylobacter* isolates.

Purpose: To assess changes in antimicrobial susceptibilities of *Campylobacter* isolates recovered from chicken carcass rinsates at slaughter.

Methods: Chicken carcass rinsates were collected from federally inspected slaughter/processing plants by USDA's Food Safety and Inspection Service (FSIS) Pathogen Reduction: Hazard Analysis Critical Control Point (PR/HACCP) verification testing program during 2007 were submitted to NARMS. *Campylobacter* isolates were recovered from spent chicken carcass rinsates using conventional techniques and tested for minimum inhibitory concentrations using a custom panel of nine antimicrobials and following CLSI standards. Confirmation and speciation were obtained using the *Campylobacter* BAX® PCR (DuPont Qualicon; Wilmington, DE) according to manufacturer's directions.

Results: In 2007, 205 rinsates were positive for *Campylobacter* which resulted in 140 (50.7%) *C. jejuni* isolates, 64 (31.2%) *C. coli*, and 1 unspeciated. Among *C. jejuni* isolates, resistance was observed to tetracycline (TET; 55.7%), ciprofloxacin (CIP; 21.4%) and nalidixic acid (NA; 20.7%). *C. coli* isolates were resistant to all antimicrobials except florfenicol: TET (37.5%), CIP, azithromycin, erythromycin and NA (all at 17.2%) and gentamicin (1.6%). An MIC of $\geq$ 1 for florfenicol occurred more often among *C. coli* isolates than *C. jejuni* isolates (92.2% and 63.5%, respectively).

Summary: Overall, *C. coli* isolates tend to be resistant to more antimicrobial agents than *C. jejuni* isolates. In comparison with 2006 results, the most dramatic shifts were observed for TET, CIP and NA resistance in *C. jejuni* (56.1%, 8.3% and 8.8%, respectively).

Significance: These data demonstrate the likelihood that the breakpoint for NA requires further evaluation. Additionally, continued monitoring and studies are required to determine why the shift in resistance has occurred.

T2-09 Modeling of Activity of Triple Combinations of Antimicrobials, Using Lauric Arginate, Cinnamic Acid, and Sodium Benzoate or Potassium Sorbate as a Case Study

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Introduction: Interest in the use of combinations of antimicrobials to improve food quality and safety in the food industry has increased due to the need to replace existing or develop new preservation systems. Currently, there is a lack of understanding of how to identify synergistic activities between multiple antimicrobials.

Purpose: The purpose of this study was to quantify and model the antimicrobial efficacy of triple combinations of lauric arginate (LAE), cinnamic acid and Na-benzoate or K-sorbate against four spoilage yeasts to determine potential synergistic, additive or antagonistic activities between the antimicrobials.

Methods: A microtiter assay was used to assess the inhibitory abilities of combinations of LAE, cinnamic acid and Na-benzoate or K-sorbate against *Saccharomycese cereviseae* (SC), *Zygosaccharomycese bailii* (ZB), *Brettanomycese bruxellosis* (BB) and *Brettanomycese narrdensis* (BN) at pH 3.0, 3.5 and 4.0 by inoculating cultures into microtiter wells containing agent combinations. Plates were incubated at 25°C for 7 days and optical density (OD) measured to assess growth. Critical concentrations (ccrit) were calculated by interpolation from single sets of OD versus antimicrobial concentration data, where OD = 0.05 (the current definition of the minimum inhibitory concentration), and then were plotted versus concentrations of the other two antimicrobials to assess interactions between the three antimicrobials.

Results: For SC, ZB and BB, combinations of organic acids (e.g., Na-benzoate or K-sorbate with cinnamic acid) showed simple additive behavior in terms of inhibitory efficacies; that is, the concentration of one of the organic acids required to inhibit growth (ccrit) decreased linearly with increasing concentration of the second organic acid. Addition of up to 15 ppm LAE introduced strong synergistic activities, i.e., ccrit decreased exponentially with addition of LAE regardless of strain. For example, for ZB at pH 3.0, ccrit of Na-benzoate ranged between 400–350 ppm when combined with 10–20 ppm cinnamic acid, while after addition of 5 ppm LAE, ccrit decreased from 300 ppm to 80 ppm. For BN, both combinations of organic acids and combinations of organic acids with LAE acted synergistically to inhibit growth, suggesting that the strain was more sensitive to organic acids than were the other tested strains.

Significance: Results showed that (1) triplet combinations of LAE, cinnamic acid, Na-benzoate or K-sorbate demonstrated high synergistic activities and (2) evaluation of the concentration dependence of ccrit may be a powerful mean to identify interactions between multiple antimicrobials in terms of their efficacy.

T2-10 DSC Inactivation of *Salmonella* spp. and *Escherichia coli* O157:H7 on Tomatoes by Allyl Isothiocyanate, Carvacrol and Cinnamaldehyde in Vapor-State

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Introduction: Antimicrobials in solution have been studied as surface treatments to control pathogens on tomatoes. However, vapor-phase antimicrobials may be more effective against surface-attached and internalized pathogens.

Purpose: We determined the activity of various volatile antimicrobials in the vapor state against pathogens on tomatoes.

Methods: Salmonella spp. and *E. coli* O157:H7 on sliced and whole tomatoes were treated in a 120 ml sealed container with various concentrations of allyl isothiocyanate (AIT), cinnamaldehyde and carvacrol in vapor-form. The samples were incubated at 4 and 10°C for up to 10 days and at 25°C for up to 10 h.

Results: AIT exhibited the greatest inactivation against the pathogens on sliced and whole tomatoes, followed by cinnamaldehyde. The lowest level of AIT (1 µl/120 ml volume) inactivated salmonellae on sliced tomatoes by 1.0 and 3.5 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. This level of AIT inactivated salmonellae on whole tomatoes by 1.5 and 2.2 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. AIT also inactivated *E. coli* O157:H7 on sliced tomatoes by 4.0 and 3.0 log at 4 and 10°C, respectively, in 10 days, with no inactivation at 25°C in 10 h. AIT reduced *E. coli* O157:H7 on whole tomato surface by 3.0 and 1.0 log CFU at 4 and 10°C, respectively, in 10 days and by 2.0 log CFU at 25°C in 10 h. Greater inactivation occurred for all treatments at 10 than at 4°C. Pathogens on sliced tomato were not inactivated at 25°C.

Significance: Antimicrobials in vapor form may be useful for controlling pathogens on fresh tomatoes marketed in packages containing head space.

T2-11 Survival of *Yersinia* in Whole Liquid Egg as Influenced by the Presence of Nisin

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Introduction: Yersinia is a psychrotrophic, gram-negative bacterium capable of causing foodborne illnesses. The bacteriocin nisin, traditionally used to inhibit gram-positive bacteria, may be bacteriostatic to gram-negative bacteria under certain conditions. Nisin may be used at levels of up to 15 µg/g in the processing of liquid egg products in the United States, although its influence on the survival of *Yersinia* in liquid egg is unknown.

Purpose: A study was conducted to determine the effects of nisin on the survival of *Yersinia* in whole liquid egg (WLE) stored at refrigerated and abused temperatures.

Methods: Four strains of *Yersinia* were inoculated into WLE at a population of ca. 4.40 log CFU/ml and acclimated for two hours before the addition of nisin at concentrations of 0, 15, 150 and 500 IU/ml, stored at 5, 10, and 21°C, and sampled at hours 0, 24, 48, and 72. Data from three experimental replications were pooled and statistically evaluated.

Results: There were no significant differences ($P > 0.05$) in populations of *Yersinia* in WLE based on variations in nisin concentration. Populations stored at 5°C increased significantly by each consecutive sampling time at hours 24 and 48 (4.79 and 4.96 log CFU/ml), but not at hour 72 (5.01 log CFU/ml). *Yersinia* populations increased significantly at each sampling time when stored at 10°C, from 4.43 log CFU/ml (hour 0) to 5.15, 5.94 and 6.60 log CFU/ml at 24, 48, and 72 hours, respectively. When WLE was stored at 21°C, *Yersinia* increased to 5.51 and 8.70 log CFU/ml at 24 and 48 h of storage, respectively, although populations declined to 8.29 log CFU/ml by hour 72.

Significance: These results indicate that the presence of nisin at concentrations up to 500 IU/ml does not influence the growth or survival of *Yersinia* in WLE stored at 5, 10, or 21°C for up to 72 hours.

T2-12 DSC Effects of Protein and Fat on Viral Inactivation through High Pressure Processing in Seafood Salad

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Introduction: High pressure processing (HPP) is a novel technology that can inactivate foodborne pathogens, including viruses. Conformational changes in the viral capsid structure caused by HPP can affect viral stability and influence the infectivity of the virus. Various components of food such as protein, fats and carbohydrates have the ability to protect virus from inactivation by HPP.

Purpose: This study assessed the protective effects of various food components (fats and proteins) on viral inactivation through HPP. The ingredient building of a seafood salad was used to observe possible protective effects.

Methods: Seafood (cod, shrimp, tuna and clams) as well as seafood with mayonnaise were inoculated with Hepatitis A Virus (HAV), Feline calicivirus (FCV), and Mouse Norovirus (MNV). Samples were pressure treated at 400, 250 and 200 MPa at 5°C for 5 minutes. Viruses were recovered with virus elution buffer (3% beef extract, pH 9.6) and titer was determined with TCID50 (tissue culture infectious dose for 50% of the cultures), using the Reed Muench equation.

Results: Viral inactivation was impacted by both the physical and chemical composition of the food. At 400 MPa, HAV inoculated tuna had a 4.33 ± 0.71 log inactivation, however, upon the addition of mayonnaise, only a 3.30 ± 0.13 log inactivation was seen, indicating the significant role fat plays in viral inactivation. At 200 MPa, FCV inoculated cod had a 1.15 ± 0.5 log inactivation compared to other seafood meats (3.15 ± 1.5 to ~4.5 log inactivation), showing the protective effects of protein concentration on HPP. Similarly, protein content of shrimp affected viral inactivation on MNV, since only ~1.8 logs were inactivated at 200 MPa.

Significance: The ability of HPP to inactivate foodborne viruses greatly depends on the composition of the food. Results indicated that at high pressures, fat alters the inhibition of viruses. Protein composition of the food also plays a large role in the resistance to pressure.

T3-01 DSC Development of Best Management Practices to Reduce the Likelihood of Soybeans Accumulating High Levels of Cadmium

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Introduction: Exposure to cadmium (Cd) has been associated with kidney dysfunction, osteoporosis, and cancer in humans. The pathway to humans is through smoking and the consumption of high-risk foods, mainly leafy greens, root vegetables and cereal crops. North Americans at greatest risk are those with nutritional deficiencies, specifically anemia and/or hypocalcemia, and infants, because of their immature gut. The accumulation of Cd in crops is not a straightforward outcome of the Cd level in soil, with soil and cultivar characteristics playing an important role.

Purpose: The objectives of this research are to identify the most influential soil characteristics among known soybean cultivars that influence Cd accumulation. The focus on soybeans is a result of the Total Diet Health Study by Health Canada identifying soy-based infant formula as having greater than 2 times the level of Cd compared to dairy-based infant formula.

Methods: Four cultivars, two of which have previously been shown to accumulate Cd at low levels and the other two at high levels, were assessed at two Ontario sites during year one and at five Ontario sites during year two. Soils were analyzed for factors previously known to influence Cd bioavailability to soybean roots: total and extractable Cd, Ca, Zn, Mg, Cl, $CaCO_3$, soil pH, organic matter and clay content.

Results: Results indicate that soil pH significantly influenced Cd accumulation in soybeans, with anions, competing cations, organic and inorganic ligands having a lesser influence. Plant genetics is a key factor in moving the Cd from the roots to the edible portion of the plant, and this work confirmed that heritable and relative differences among cultivars dictate the degree of Cd accumulation that is initially dependent on soil characteristics.

Significance: This information will be used to develop best management practices for soybean farmers, to reduce the likelihood of soybeans exceeding the CODEX guideline of 0.2 ppm for Cd.

T3-02 DSC Analysis of the Mycotoxin Zearalenone and Masked Mycotoxins α- and β-Zearalenol Glucoside in Wheat Samples, Using a New LC-UV/MS Method

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Introduction: Masked mycotoxins are conjugates of mycotoxins such as zearalenone (ZEN) and analogues α- and β-Zearalenol (ZOL). Conjugates α- and β-ZOL-glucosides (glu) are not routinely analyzed in food, yet they contribute to total mycotoxin contents. Liquid chromatography (LC) with Ultraviolet (UV) /mass spectrometry (MS) detection can be used to assess risk of exposure to these conjugates.

Purpose: The purpose of this study was to analyze ZEN and ZOL-glu in wheat, using a new LC-UV/MS method and laboratory-prepared silica cleanup columns.

Methods: Wheat spiked with ZEN at 3 levels (0.5, 1 and 5 µg/g; n = 15) and the conjugates at 0.5 and 1 µg/g (n = 6) were subjected to the new method, which included sample centrifugation in methanol:methylene chloride (50:50, v/v), cleanup through the columns and separation/detection by LC-UV/MS (C18 column; 70% methanol:water isocratic mobile phase; ESI scan). Field wheat samples (n = 51) were analyzed without spiking.

Results: Mean ZEN recoveries ranged from 100 (± 1.98) to 105 (± 6.35) % while mean β-ZOL-glu and β-ZOL-glu recoveries ranged from 76.7(± 12.9) to 93.6 (± 18.7) % and 51.3 (± 1 0.41) to 83.53 (± 16.51) %, respectively. Wheat samples contained ZEN (0.34 ± 1.47 µg/g), β-ZOL-glu (0.17 ± 0.91 µg/g) and β-ZOL-glu (0.06 ± 0.27 µg/g) while percentage levels above 0.5 µg/g were 13, 5.9 and 1.96% for ZEN, β- and β-ZOL-glu, respectively. Zearalenone content was not significantly different than β-ZOL-glu and β-ZOL-glu ($P = 0.48$; $P = 0.19$, respectively); β- and β-ZOL-glu contents were not different ($P = 0.423$). Limits of detection (UV/MS) for ZEN, β-and β-ZOL-glu were: 2 ng/kg/1 ng/kg; 0.5 µg/kg/20 ng/kg; 0.5 µg/40 ng/kg, while corresponding limits of quantification were 0.2 µg/kg/10 ng/kg; 1 µg/kg/0.1 µg/kg; 5 µg/kg/0.2 µg/kg, respectively.

Significance: This new LC-UV/MS method detected mycotoxins β- and β-ZOL-glu and ZEN in wheat at levels about the legal limit. Laboratory-made columns and common analytical tools can be used to analyze parent and masked mycotoxins for risk assessment.

T3-03 Decrease in the Mercury Concentration in Bluefin Tuna by Breeding Fishes with Low Mercury Levels

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Introduction: We have previously reported that the total mercury concentration in cultured bluefin tuna does not correspond with body weight and its upper limit is about 0.6 ppm; this observation is in contrast to that in wild tuna. We speculated that breeding food, the same size mackerel, even after growing of bluefin tuna is complete, might be the cause of lack of positive correlation between body weight and mercury concentration.

Purpose: In the present study, we attempted to decrease the mercury concentration in bluefin tuna by raising fish with lower mercury concentrations.

Methods: We raised bluefin tuna (1 year after hatching) in 2 net cages. The control group was raised on spotted mackerel whose mercury concentration was 0.052 ppm. On the other hand, the test group was raised on sand lace and horse mackerel, whose mercury concentrations were 0.037 and 0.016 ppm, respectively. The breeding continued for 18 months, and the total mercury concentration of 7 parts of muscle and internal organs was determined by the cold vapor atomic absorption method.

Results: In the control group, after 18 months, body weight was 33 kg; the mercury concentration of the muscle was 0.6 ppm, which is consistent with the value observed in the previous study. However, in the test group, whose body weight was 25 kg, the concentration was maintained at 0.3 ppm and it showed no increase during the experiment irrespective of increase of body weight. The concentration in the internal organs was also lower in the test group.

Significance: We could reduce the mercury concentration in cultured bluefin tuna by raising them on horse mackerel and sand lace. The mercury level in the cultured bluefin tuna was less than the Japanese standard values. We could develop an important breeding method to lower the threat of mercury toxicity.

T3-04 DSC A Comparative Study for the Detection of Histamine-Producing Bacteria in Fish by Culture, Potentiometric and Molecular-Based Methods

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Introduction: Detection of histamine-producing bacteria in fish is commonly performed using a modified Niven's agar method known to result in a high number of false positives and negatives. In our study, Niven's positive isolates were tested against potentiometric and PCR-based detection methods. These three methods were compared with bacterial histamine production confirmed using a HPLC diode array method.

Purpose: To establish a convenient and more reliable screening method for the detection of histamine-producing bacteria in fish.

Methods: Tuna, mahi-mahi, wahoo and bluefish surface swab and tissue samples were enriched in histidine-containing broths at 37°C for 24 h prior to plating on TSA (2% NaCl); representative colonies were streaked on Niven's agar. Positive strains on Niven's agar were tested for an increase in conductance with histidine broth (pH 5.5, 30°C) and for the presence of the histidine decarboxylase (hdc) gene by PCR amplification of a 709 bp fragment. Bacterial histamine production was compared to results using detection methods based on HPLC after 48 h incubation at 37°C in TSB containing 1% histidine, 2% NaCl, and 0.0005% pyridoxal-HCl (pH 6.5).

Results: Of 124 Niven's positive isolates, 58% of strains were high histamine producers (> 1,000 ppm), 2% were moderate producers (250–1,000 ppm), 22% were low producers (< 250 ppm) and 19% produced no detectable amount of histamine (< 25 ppm). All high histamine-producing strains (71/71) exhibited an increase in conductance in histidine broth and were positive for the presence of the hdc gene. Low and no detectable histamine-producers did not show either an increasein conductance or the presence of the hdc gene (0/51).

Significance: Recent molecular-based methods are more reliable than currently used culture-based methods for detection of high histamine-producing bacteria in fish. The PCR-based detection method can be used to screen for the presence of histamine-producing bacteria in fish prior to formation of high histamine levels.

T3-05 Effect of Cooling and Temperatures on Quality and Safety of Quahog Clams (*Mercenaria mercenaria*)

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Introduction: The Model Ordinace, NSSP Guide for molluscan shellfish, requires shellstock to be cooled to 50°F and shucked shellfish to 45°F within varying time periods of 36, 24, 20, 12, or 10 h. The Model Ordinance was established for oysters; however, the regulation has been applied to clams even though there is a lack

of scientific information or health statistics for the inclusion of clams.

Purpose: The objective of this study was to determine whether two alternative cooling temperatures and times would result in clams having reduced mortalities while providing a microbially safe product to consumers. The standard now is to harvest clams and leave no longer than 20 h, before storage at 45°F. The first alternative was to harvest clams and hold no more than 5 hrs, then to 65°F for 12 h, then to 55°F for 12 h and finally to 45°F. The second alternative was to harvest clams and hold no more than 5 h, move to 55°F for 24 h, and then finally to 45°F.

Methods: A monitored thermocouple was inserted into the growing bed two weeks before harvest, so that the clams could be harvested when the water temperature was equal to or higher than 80°F. 2000 clams per treatment were processed according to the temperature schemes listed in the purpose section. Live clams were analyzed for their aerobic plate count on days 0, 1, 7, 14, and 21.

Results: There is a statistically significant difference ($P < 0.05$) between the standard and the two alternative methods. The overall aerobic plate count mean for the standard method is 2.3×10^6 compared to counts with the two alternative methods, 9.9×10^5 and 6.6×10^5 for the second and first alternatives respectively.

Significance: The data suggests that the clam industry should evaluate the current cooling rates and temperatures used when harvesting.

T3-06 DSC Changes in the Levels of *Vibrio parahaemolyticus* and *V. vulnificus* during Commercial Harvesting of Gulf Coast Oysters

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Introduction: Molluscan shellfish harvesting guidelines stipulate refrigeration within a defined time based on harvest season. Nonetheless, even the more stringent summer guidelines allow for extended storage of oysters at ambient temperatures, providing an opportunity for bacterial proliferation.

Purpose: The purpose of this study was to evaluate the impact of on-deck storage on the levels of *V. vulnificus* and *V. parahaemolyticus* in commercially harvested oysters.

Methods: Oysters were harvested seasonally from 3 US Gulf Coast sites over 2 years. Samples were taken at the beginning of harvest and 2.5, 5.0, 7.5, and 10 h after holding at ambient conditions on the boat deck. Chilled oyster samples were processed for enumeration of *V. parahaemolyticus* and *V. vulnificus* using colony lift hybridization (tlh and vvhA gene targets, respectively). Representative *V. vulnificus* isolates were subjected to genotyping. Statistical relationships were established by ANOVA.

Results: Ambient air temperatures were 10–15°C, 16–21°C, and 26–29°C for winter, spring/fall, and summer, respectively. During summer, increases in *V. vulnificus* and *V. parahaemolyticus* were as high as 1.60 $\log_{10}$ CFU/g after 10 h storage; in spring/fall oysters, a 1.0 $\log_{10}$ increase was observed; and in winter, increases were < 0.5 $\log_{10}$. Statistically significant ($P < 0.05$) correlations between ambient air temperature and increases in total *V. parahaemolyticus* and *V. vulnificus* counts were noted. Summer *V. vulnificus* isolates contained a higher percentage (40%) of genotype B strains, which are more often associated with clinical illnesses. Spring/fall isolates were more often genotype A (80%), associated with environmental origin.

Significance: While increases in *V. parahaemolyticus* levels were consistent with previous estimates, those for *V. vulnificus* were higher than expected. The study provides information about how commercial harvesting conditions impact the levels and/or strain types of *V. vulnificus* and *V. parahaemolyticus* in Gulf Coast oysters. These data are relevant to future risk assessment iterations and for informing policy decisions.

T3-07 DSC Molecular Characterization of Antibiotic Resistant *Salmonella* Typhimurium and *Salmonella* Kentucky Recovered from Pre- and Post-Chill Whole Broiler Carcasses

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Introduction: Salmonella causes 1.4 million cases of salmonellosis and over 500 deaths annually in the United States. Salmonellosis is mainly associated with food of animal origin. Antibiotic resistance increases the mortality rates due to foodborne illness, and has been linked to the overuse or misuse of antibiotics in agriculture and in human and veterinary medicines. Little information is available about the molecular characterization of *S.* Typhimurium and *S.* Kentucky recovered from pre- and post-chill whole broiler carcasses.

Purpose: The purpose of this study was to screen class-I integron and BlaCMY resistance gene and to characterize genetic diversity of *S.* Typhimurium and *S.* Kentucky recovered from pre- and post-chill broiler carcasses by molecular methods.

Methods: In this study 218 sulfisoxazole- and ceftiofur-resistant *S.* Typhimurium and *S.* Kentucky isolates were tested for class-I integrons and BlaCMY genes by PCR. Then pulsed field gel electrophoresis (PFGE) was performed according to the Centers for Disease Control and Prevention protocol to determine the genomic DNA fingerprinting profiles of the isolates.

Results: One hundred and forty (93%) ceftiofur-resistant *S.* Kentucky isolates had BlaCMY genes. However, no class-I integrons were detected in sulfisoxazole-resistant isolates. On the other hand, 43 (91%) ceftiofur-resistant *S.* Typhimurim isolates had BlaCMY genes, and four (5.5%) contained class-I integrons in sulfisoxazole-resistant isolates. Twenty-nine PFGE patterns were detected with XbaI among 67 *S.* Typhimurium isolates, and 38 patterns among 151 *S.* Kentucky isolates.

Significance: The results suggest that class-I integrons and blaCMY genes contribute to specific antimicrobial resistance among *S.* Typhimurium and *S.* Kentucky recovered from pre- and post-chill whole broiler carcasses. The research also indicates that PFGE reveals a genetically diverse population within the recovered isolates.

T3-08 Validation of Intervention Strategies to Control *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 in Moisture-Enhanced Beef

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Introduction: Since 2000, three different outbreaks have been linked to the consumption of mechanically tenderized meat products contaminated with *Escherichia coli* O157:H7. Following this, all establishments producing non-intact beef products were required by the USDA-FSIS to reassess their HACCP plans because of potential contamination risk to consumers.

Purpose: The purpose of this study was to determine the effectiveness of lactic acid producing bacteria (LAB; ~10^7 CFU/g), acidified sodium chlorite (ASC; 1000 ppm), and lactic acid (LA; 3%) as intervention strategies to control *E. coli* O157:H7 and *Salmonella* in moisture-enhanced beef.

Methods: For each pathogen, samples were inoculated and treated with one of the intervention methods. After the age period (14 or 21 days) samples were subjected to enhancement. Surface and internal counts were enumerated by plating on an overlay of MacConkey Agar and Tryptic Soy Agar (*E. coli* O157:H7) and xylose lysine decarboxylase and Tryptic Soy Agar (*Salmonella*) and incubated overnight at 37°C.

Results: On day 21, all interventions were effective ($P < 0.05$) in reducing *E. coli* O157:H7 surface counts. On day 14, LA reduced internal counts by > 1.7 logs ($P = 0.03$). On day 21, all interventions were effective in reducing internal *E. coli* O157:H7 counts ($P < 0.05$). *Salmonella* surface counts were significantly reduced by the application of all the interventions at day 14 and by day 21 for LA and LAB treatments ($P < 0.05$). Internal *Salmonella* counts were reduced by 1.5 logs by spraying LAB and LA on day 14 and by 2.0 logs after application of LA on day 21.

Significance: This study validates the application of LAB, ASC, and LA sprays as effective interventions to reduce pathogenic counts in moisture enhanced / brine injected beef subprimals. Processors can use this data for HACCP plan reassessment as mandated by the USDA-FSIS.

T3-09 DSC Validation of Commercial Thermal Process for Control of *Escherichia coli* O157:H7 and *Salmonella* spp. in Chopped and Formed Beef Jerky

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Introduction: The United States Dept. of Agriculture/Food Safety and Inspection Service (USDA/FSIS) issued "Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants" because of a salmonellosis outbreak linked to beef jerky.

Purpose: The objective of this study was to determine the efficacy of a worst-case scenario, commercial thermal process (CP) for controlling pathogens during processing of chopped and formed beef jerky.

Methods: Escherichia coli O157:H7 and *Salmonella* spp. were inoculated into raw chopped and formed jerky batter (ca. 7.0 and 5.5 log CFU/g, respectively) and then extruded into 2.54 cm by 0.64 cm by 15.24 cm strips and thermally processed for 6.75 h in a commercial smokehouse. The CP consisted of 45 min at 52°C dry bulb (DB) and no humidity, 1 h at 57C DB [20% relative humidity (RH)], 60C DB (23% RH) for 45 min, and 63C DB (22% RH) for 45 min. A drying cycle (DC) of 1.5 h at 68°C DB (no humidity) and 2 h at 77°C DB (15% RH) was added following the CP.

Results: A 5-log reduction was observed for *Salmonella* spp. populations on xylose lysine desoxycholate agar at the end of CP; however, CP alone produced only a 3.8-log reduction of *E. coli* O157:H7 populations on phenyl red sorbitol agar. An additional 1.5 h was needed to achieve a 5-log reduction of *E. coli* O157:H7. Water activity and moisture-to-protein ratio (MPR) values were 0.792 and 1.27:1, and 0.603 and 0.82:1 at the end of the CP and DC, respectively.

Significance: The worst-case scenario for a commercial jerky process does not achieve the 5-log reduction of both pathogens as required by FSIS, but additional drying at 68°C (DB) for 1.5 h achieved 5-log reductions of both pathogens. Further drying would be needed to meet the MPR of 0.75:1 per FSIS beef jerky requirements.

T3-10 FSIS Microbiological Testing Program for *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products, 1994–2006

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Introduction: The US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has tested for *Listeria monocytogenes* (*Lm*), a pathogen of great concern with a high mortality rate, in Ready-to-Eat (RTE) meat and poultry since 1987. This program helps protect public health by detecting positive products that may cause foodborne illness and removing them from commerce.

Purpose: To present trends of *Lm* from regulatory sampling from 1994–2006.

Methods: Random samples collected from establishments were shipped overnight to FSIS laboratories. Initially, > 1 lb samples were collected for most products, increasing to >2 lbs in 2001, and 25 g samples were analyzed. Testing was performed according to FSIS Directives and Microbiological Laboratory Guidebook.

Results: From 1994–006, a total of 1,142/87,764 (1.30%) of routine samples from 4,024 establishments were positive. A decreasing trend was seen from 85/2,935 (2.89%) in 1994 to 60/12,372 (0.48%) in 2006. Positives were higher during the summer 322/21,611 (1.49%) and spring 322/23,452 (1.37%) than the fall 293/22,160 (1.32%) and winter 205/20,541 (1.00%) seasons. Levels were also higher in the Southeast 233/13,116 (1.78%), than in the North Central 243/22,025 (1.10%), West 216/17,371 (1.24%), Northeast 286/18,780 (1.52%), and Southwest 164/16,472 (1.00%) regions. Higher levels of positives were observed in sliced ham and luncheon meats from 1994–2000, 126/3,054 (4.13%), and sliced, diced, and shredded products from 2001–2006, 144/12,269 (1.17%). From the follow-up sampling, generally conducted as a result of finding positives, a total of 576/8,903 (6.5%) samples from 1,045 establishments tested positive.

Significance: As programs have evolved from 1994–2006, changes in sample collection and analysis have made statistical comparison invalid; however, the aggregate data provide an overall indication of trends. *Lm* trends show decreases in percent positives concurrent with changes in FSIS policies and increased industry response to these policies.

T3-11 On-Line Brush and Spray Washers to Lower Numbers of *Campylobacter* and *Escherichia coli* and Presence of *Salmonella* on Broiler Carcasses during Processing

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Introduction: Physical and chemical procedures are applied to broiler carcasses during processing to remove bacteria. It is unclear how effective different types of wash steps are in lowering the presence or numbers of pathogenic bacteria.

Purpose: The objective of this study was to measure the individual and combined effectiveness of five separate on-line wash steps applied between exsanguination and chilling in a commercial US broiler processing plant.

Methods: In each of five replicate plant visits, five carcasses were collected from the shackle line directly before and after each of five separate wash steps. Sample sites included: pre-scald brush washer, post feather pick (New York dressed) spray washer, inside/outside spray washer, post-evisceration brush washer and final pre-chill spray washer. Carcasses were rinsed in PBS; rinsate was examined for numbers of *Campylobacter* and *E. coli* and presence of *Salmonella,* using standard cultural methods. Numbers were compared using Tukey's honest significant difference test; prevalence was compared using Kruskall-Wallis test.

Results: Overall, numbers of *Campylobacter* were lowered from 2.58 log to 1.15 log CFU/ml carcass rinse but the inside/outside washer was the only step that caused a significant drop by itself. Although no single wash step caused a significant decrease in *E. coli* or *Salmonella*, the five wash steps in series did lower *E. coli* numbers (from 4.60 log to 2.69 log CFU/ml) and reduced *Salmonella* prevalence (from 80% to 24%).

Significance: Although the benefit of broiler carcass wash steps when examined in isolation may not be evident, when these steps are combined in series they are effective in lessening bacterial contamination on carcasses.

T3-12 Multiplication of *Salmonella* Enteritidis on Egg Yolk Membranes and Penetration into Yolk Contents

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Introduction: Prompt refrigeration to limit bacterial multiplication is a critical aspect of efforts to control the transmission of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) to consumers of contaminated eggs. However, a proposed national *S.* Enteritidis control program would allow unrefrigerated ambient temperature storage of eggs on farms for up to 36 h. Although the nutrient-rich yolk interior is an uncommon location for *S.* Enteritidis contamination in freshly laid, naturally contaminated eggs, migration across the vitelline membrane could lead to rapid bacterial multiplication inside eggs even when the initial site of deposition is outside the yolk.

Purpose: The objective of the present study was to determine whether bacterial multiplication can also occur on the egg yolk membrane (before or in addition to multiplication within the yolk contents) and thereby further increase the risk for consumers.

Methods: Using an in vitro egg contamination model, four strains of *S.* Enteritidis were inoculated onto the exterior surface of yolk membranes, and their ability to either multiply in association with the yolk membrane or migrate through that membrane to reach the yolk contents was assessed during 36 h of incubation at 30°C.

Results: All four *S.* Enteritidis strains penetrated the vitelline membrane to reach the yolk contents (at an overall frequency of 11.5%) during the first 12 h of incubation. The mean $\log_{10}$ concentration of *S.* Enteritidis was significantly higher in whole yolks (including yolk membranes) than in yolk contents at both 12 h (0.818 vs. 0.167 CFU/ml) and 36 h (2.767 vs. 1.402 CFU/ml) of incubation.

Significance: These results demonstrate that *S.* Enteritidis multiplication on the vitelline membrane may both precede and exceed multiplication resulting from penetration into the yolk contents during the first 36 h of unrefrigerated storage, further documenting the importance of rapid refrigeration for protecting consumers from egg-transmitted illness.

T4-01 A Chain Modeling Approach to Estimate the Impact of Soil Cadmium Pollution on Human Dietary Exposure

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Introduction: Cadmium in soil poses a risk for human health because of its accumulation in food and feed crops. The extent of accumulation strongly depends on soil type and the degree of pollution.

Purpose: The objective of the present study was to develop a supply chain model to estimate long term human dietary cadmium exposure from (regional) soil characteristics.

Methods: Six soil scenarios were assessed, reflecting a specific contaminated region: a) 0.5 mg kg-1 Cd / pH 4.5, b) 0.5 mg kg-1 Cd / pH 5.5, c) 1.0 mg kg-1 Cd / pH 4.5, d) 1.0 mg kg-1 Cd / pH 5.5, e) 2.5 mg kg-1 Cd / pH 4.5 and f) 2.5 mg kg-1 Cd / pH 5.5. Cadmium levels in feed crops, vegetables and cattle kidneys/livers/meat were estimated with regression and mathematical models. Human exposure was estimated by Monte Carlo simulation, using a consumption database. The national exposure, based on national monitoring data, was used as a reference. The exposure based on measured contamination levels in food products produced in the specific region was used for validation.

Results: The national exposure was 0.16 µg kg bw-1 day-1, with 0.25% of the population exceeding the TDI of 0.5 µg kg bw-1 day -1. The exposure for scenario a to f was 0.33, 0.24, 0.50, 0.36, 0.98 and 0.63 µg kg bw-1 day-1. The TDI was exceeded by 10%, 3%, 50%,

25%, 80% and 60% of the regional population. The validation exposure was 0.28 µg kg bw-1 day -1, with10% of the regional population exceeding the TDI, and was between the two most realistic regional soil scenarios (a and b).

Significance: The model can be used for fast evaluation of dietary cadmium exposure and the identification of risk areas based on soil conditions. The model can be easily applied to other contaminants and/or crops.

T4-02 Risk-Based Sampling for Foodborne Pathogens — A Probabilistic Algorithm for *Escherichia coli* O157:H7 Sampling of the Federally Inspected Ground Beef Supply

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Introduction: The Food Safety and Inspection Service (FSIS) has tested for the presence of *Escherichia coli* O157:H7 in the raw ground beef produced by federally inspected establishments since 1994. Because all establishments had an equal probability of being selected for sampling, FSIS resources were allocated uniformly among establishments that differed greatly in terms of their potential exposure of consumers to *E. coli* O157:H7. Here we describe a risk based sampling algorithm— both the statistical/computational design and the components of risk it will consider.

Purpose: The risk-based sampling algorithm was developed to help the Food Safety Inspection Service allocate resources more effectively and better protect public health.

Methods: A probabilistic algorithm that selects establishments for sampling was developed in Visual Basic using Microsoft Excel.

Results: The algorithm allocates samples in a random draw, where the probability of each establishment being sampled can be weighted by virtually any number of factors. The source code weights the probability of each plant being selected according to (Volume*Hazard/sum of all Volume*Hazard). Currently, the algorithm weights sampling probability according to production volume (Scaled according to a user input value and the relationship of (v = SL+((vi-v4)/(v1-v4/SH-SL) while the increased risk calculated for positive test results is handled through an external "follow-up" sampling program (Odds ratio: 4.86; 95% confidence interval: 3.17–7.46). The algorithm code also allows management to input an upper and lower bound of samples. Given the current upper bound of 2/month and a lower bound of 3 samples per year, the expected sampling frequencies of plants with no positive FSIS samples in the last 120 days is as follows:

< 1,000 pounds	0.63
1,000 to 50K pounds	0.99
>50K to 250K pounds	1.98
> 250K pounds	1.94

Significance: The risk-based sampling algorithm allocates samples based on the potential for consumer exposure to *E. coli* O157:H7. The algorithm was developed to be readily expanded to include risk factors as FSIS becomes aware of them and data is made available. This risk-based sampling approach increases both the efficiency of government resource allocation and the public health impact of the sampling program.

T4-03 Risk Assessment for Thermal Inactivation of *Salmonella* spp. in Fresh Pork

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Introduction: Published literature indicates that temperatures destroying *Salmonella* spp. will also destroy other pathogens in fresh pork.

Purpose: The aim of this risk assessment was to evaluate the risk of salmonellosis from consuming moisture enhanced and un-enhanced pork meats and changes in risks when different cooking "endpoint" temperatures (145 – 160°F) at the geometric center of the product is achieved.

Methods: A retail-to-table probabilistic risk assessment was developed with two components: exposure assessment and illness/risk characterization. The exposure assessment begins with estimating distributions of *Salmonella* on pork meats at retail, using data from a NPB survey. The exposure model considers growth in levels during transportation from retail to homes and storage/refrigeration in homes, and decline in levels from thermal inactivation before consumption. Audits International data on transportation duration/temperature and RTI survey data on consumer practices were used. The risk characterization combined simulated exposure with dose-response developed by the FAO/WHO to estimate risk per serving. FSIS carcass prevalence data was used as an upper-bound distribution of levels on pork at retail in a sensitivity analysis.

Results: At the upper percentiles (> 99.9th), representative of extreme temperature abuses, growth during storage/refrigeration were > 6 log CFU/g. However, with thermal treatment achieving an endpoint temperature of 160°F (baseline scenario), there is no surviving *Salmonella* at all percentiles. With alternative endpoint temperatures (145, 150, 155°F), risks per serving were also zero, except for extreme temperature abuses. At the upper percentiles corresponding to extreme temperature abuses, predicted levels of *Salmonella* spp corresponded to risks of 10^{-4} (99th) to 10^{-2} (99.99th) per serving for some cuts. Even under the upper-bound scenario, surviving *Salmonella* were predicted only under extreme temperature abuses (> 99th).

Significance: If extreme temperature abuse during retail and consumer handling are avoided prior to consumer cooking, pork meats can be cooked to 145°F for 15 seconds without increased risk of salmonellosis.

T4-04 Evaluating the Safety of Eggs

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Introduction: Salmonella Enteritidis (SE) is not endemic in Australia; however, other *Salmonella* serovars are responsible for outbreaks of foodborne illness linked to the consumption of eggs.

Purpose: A risk assessment was undertaken to inform the development of a through-chain Australian standard for the production and processing of eggs.

Methods: Epidemiological and surveillance data were thoroughly reviewed as part of the risk assessment. In addition, steps along the production and supply chain that affect the likelihood and/or levels of *Salmonella* contamination were examined. The assessment utilized an industry-developed quantitative risk assessment model, which considered the fate of *Salmonella*

spp. in eggs from point of lay through to consumption. Importantly, it predicted the effect of storage time and temperature on the risk of illness.

Results: Consumption of well-cooked eggs (or cooked foods containing egg) presents little risk of salmonellosis, as cooking results in > 12-$\log_{10}$ inactivation of *Salmonella* spp. However, consumption of uncooked or lightly-cooked foods containing raw egg represents a potential risk. The quantitative model predicted that consumption of uncooked food containing raw eggs, stored under conditions that permit growth of *Salmonella* spp., increases the risk of illness. Unfortunately, there is limited data on actual exposure of consumers to foods containing uncooked or under-cooked eggs.

For non-SE *Salmonella* serovars the primary route of internal contamination of the egg is considered to be via transmission through the shell. The ability of *Salmonella* to migrate into the egg is influenced by many factors, including the integrity of the shell, cuticle and membranes; the presence of external contamination; temperature differentials between egg and environment; and humidity.

Significance: The risk assessment highlights production and handling factors that will require control in order to reduce food safety risks.

T4-05 DSC Predictive Modeling of *Listeria monocytogenes* Reduction on Fully-Cooked Chicken Drumettes during Post-Process Hot Water Pasteurization

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Introduction: The prevalence of *Listeria monocytogenes* in fully cooked ready-to-eat (RTE) poultry products poses potential risk of infectious diseases. Therefore, attention has been focused on the post-process pasteurization of fully-cooked poultry products.

Purpose: This research aimed to develop a pre-dictive model to assess the thermal inactivation of *L. monocytogenes* in chicken drum products during post-process hot water pasteurization.

Methods: Individual fully cooked chicken drums were surface inoculated with 7 log CFU/g of *Listeria innocua* (a non-pathogenic surrogate of *L. monocytogenes*). Inoculated samples were vacuum-packaged and treated in a hot water bath at 60, 70, 80, and 90°C for pre-determined treatment intervals.

Results: The results showed that the bacterial reductions in log CFU/g determined by standard plate count method were 4.30 after 30 min, 4.08 after 10 min, 3.54 after 6 min and 3.14 after 4.5 min at 60, 70, 80, and 90°C. A 7 log CFU/g reduction of *L.* innocua occurred at 54, 28, 18 and 10 min at 60, 70, 80, and 90°C, respectively. Weibull model was used to predict the survival of *L. innocua* at each heating temperature. The root mean square error (RMSE) of the fitted model and the plot of predicted versus observed bacterial loads indicated a good fit of the model. The established predictive model was further validated by a new data set generated in the pilot plant test at the same temperatures for different heating intervals within the modeling range. The validation showed that observed values determined with the new data fell inside the 95% pre-diction intervals of predicted values determined with the original data, indicating a good prediction of the model.

Significance: This research demonstrated that post-process hot water pasteurization is an effective microbial intervention against *L. monocytogenes* in RTE poultry products, and Weibull model can be used to predict thermal inactivation of *L. monocytogenes* in poultry products.

T4-06 Development of a Predictive Model for the Growth of *Listeria monocytogenes* in Pasteurized Vanilla Cream and Validation under Dynamic Temperature Storage Conditions

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Introduction: Listeria monocytogenes has been associated in the past few years with several food-borne illness outbreaks involving a large range of food commodities. The pathogen is of great concern for the dairy industry, as many listeriosis outbreaks have been associated with the consumption of milk and milk-based products.

Purpose: The purpose of this study was to develop and validate, under dynamic temperature conditions, a product-specific model for predicting the growth of *Listeria monocytogenes* in pasteurized vanilla cream, a traditional milk-based product.

Methods: Commercially prepared vanilla cream samples were artificially inoculated with a five strain cocktail of *L. monocytogenes* at an initial concentration of 10^2 CFU g^{-1} and stored at 3, 5, 10, and 15°C for an overall period of 36 days. The growth kinetic parameters at each temperature were determined by the primary model of Baranyi and Roberts, the modified Gompertz and the modified logistic models. The maximum specific growth rate (μmax) was further modelled as a function of temperature by means of a square root-type model. The performance of the model in predicting the growth of the pathogen at dynamic temperature conditions was based on two different temperature scenarios with periodic changes from 4 to 15°C, namely (i) 12 h at 4°C, 6 h at 8°C and 12 h at 15°C, and (ii) 12 h at 4°C and 12 h at 12°C. Growth prediction at dynamic temperature profiles was based on the square root model together with the differential equations of the Baranyi and Roberts model, which were numerically integrated with respect to time. Model performance was based on the bias (Bf) and accuracy (Af) factors, the goodness-of-fit index (Gof), and the percent relative errors (% RE) between observed and predicted growth of the pathogen.

Results: The Baranyi and Roberts model appeared to fit the overall growth data under isothermal conditions better than the modified Gompertz and modified logistic models, and it was thus used in the development of the secondary modeling approach. The developed product specific model predicted with high accuracy the growth of *L. monocytogenes* under dynamic temperature conditions. The average values for the performance indices were 1.038, 1.068, and 0.397 for Bf, Af and Gof, respectively, for both fluctuating temperature scenarios assayed, whereas more than 80% of the predictions were in the ± 10% relative error zone.

Significance: The developed product-specific dynamic model could become a useful management tool to improve the quality assurance systems in the dairy industry.

T4-07 Combined Effects of Sucrose Laurate Ester and Pressure-Assisted Thermal Processing to Inactivate *Bacillus amyloliquefaciens* Spores Suspended in Mashed Carrots

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Introduction: Combined pressure (600–900 MPa) and heat (90–121°C) treatment during pressure-assisted thermal processing (PATP) has been found to be effective in inactivating bacterial spores. Various approaches for further reducing process severity are desired.

Purpose: The objective of this study was to investigate the potential synergetic effect of sucrose laurate ester (SE) and PATP on *B. amyloliquefaciens* spores inactivation.

Methods: Spores of *B. amyloliquefacien* Fad 82 (10^8 CFU/ml) were suspended in sterile mashed carrots (MC) with or without 1.0% SE. Samples were treated at 700 MPa and 105°C for come-up time, 2 and 5 min. Heat shock (80°C, 10 min) was applied to untreated and treated samples to determine spore germination during PATP. Samples were plated on Trypticase Soy Agar enriched with 0.6% yeast extract (YE), with or without 1% SE. Most probable number technique (MPN, Trypticase Soy Broth enriched with 0.6% YE) was applied when spores were not detected in treated samples (i.e., < 10 CFU/ml). Tubes were kept at 32°C for 15 days to allow recovery of the remaining population.

Results: The combination of SE and PATP showed a synergetic effect against *B. amyloliquefaciens* spores by 0.43 to1.08 log units, depending on the holding time applied. Population of spores in MC without SE decreased to 2.20 log units, while samples with SE decreased to < 10 CFU/ml after 5 min treatment. Nevertheless, MPN results showed a recovery of 3.28 log units after the tubes were kept at 32°C for 15 days. Significant germination was detected only in MC samples treated for 2 min. When SE was added to the recovery medium, additional reduction in spore counts was observed on the untreated and PATP treated samples.

Significance: Within the experimental conditions studied, sucrose laurate ester appeared to enhance PATP spore lethality (up to 1.08 log), but showed limited effectiveness in inhibiting recovery of remaining population.

T4-08 The Role of Nutrients and Biodiversity in Controlling *Escherichia coli* O157:H7 in the Primary Production Chain of Lettuce

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Introduction: A growing number of foodborne illnesses have been associated with the consumption of fresh produce. Since various post-harvest sanitation procedures are not sufficient in removing pathogens or are not allowed, prevention of contamination is essential.

Purpose: The objective of this project was to identify risk factors for pathogen persistence in manure and soil.

Methods: (1) Manure samples from 16 organic and 9 conventional dairy farms were screened for virulence genes *stx*1, *stx*2, *eae* and rfbE (*E. coli* O157) with PCR. Subsequently, survival of *E. coli* O157:H7 (B6-914 GFP-91) was assessed, (2) *E. coli* O157:H7 survival was studied in manure from cattle fed grass/maize silage, grass silage and straw, and (3) *E. coli* O157:H7 survival in 36 soils (organic/conventional, sandy/loamy was assessed. Survival data was fitted to suitable decline models and related to chemical characteristics and microbial species richness and diversity (DGGE). Multiple regression analysis was used to identify risk factors.

Results: (1) The microbial species richness was significantly lower in rfbE positive manure (Sent = 12 ± 4) compared to rfbE negative manure (Sent = 18 ± 7) ($P = 0.009$). Survival time was predominantly determined by the number of culturable coliforms and pH (negative relations). (2) Survival of *E. coli* O157:H7 in manure was significantly reduced when cattle were fed straw (74 ± 7 days) compared to grass/maize- silage (127 ± 12) and grass-silage (98 ± 10) (all $P < 0.001$). Survival time was negatively related to pH ($r = 0.96$, $P < 0.05$) and fibre content ($P = 0.89$, $P < 0.05$). 3) Survival time in soil ranged from 54 to 105 days and was predominantly determined by the level of dissolved organic carbon per unit biomass ($R2 = 0.27$, $P = 0.0.4$). No significant differences were observed between soil and management type.

Significance: Reduction of soluble carbon compounds and mineral nitrogen in agricultural systems reduce the persistence of *E. coli* O157:H7. This can be achieved by high fiber cattle diets and regular addition of high C/N organic fertilizers to fields.

T4-09 Survival and Dispersal of Surrogate *Escherichia coli* under Lettuce Field Conditions: Effect of Irrigation

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Introduction: Field-oriented research is needed to assist industry and regulators in making science-based decisions regarding microbiological criteria for food safety of high risk crops (such as fresh leafy green vegetables) and regions.

Purpose: The purpose of this project was to determine (1) how surrogate *E. coli* persist following introduction to soil; (2) the influence of irrigation practices on survival; and (3) the behavior of nonpathogenic *E. coli* in comparative pre-trial tests with *E. coli* O157:H7.

Methods: A composite of three commensal *E. coli*, 6.0 to 9.0 log CFU per plot, were applied to soil beds prior to romaine lettuce emergence. Surface (0–2.5 cm) and sub-surface (3–8 cm; 9–13 cm) samples were collected from 0 to 28 days. Samples of run-off, roots, and lettuce were collected. Enumeration was conducted on TSA amended with 0.1% pyruvic acid, 80 mg/L rifampicin, 70 mg/L MUG, and 1.0% PCNB, incubated at 22°C for four hours, then 44.5°C for 44 h. Colonies of MUG+, rifampicin-resistant *E. coli* were purified for PFGE analysis. Run-off enumeration was conducted using Quantitray 2000 (Idexx Corp.).

Results: Survival and colonization characteristics of selected commensal *E. coli* were highly similar to *E. coli* O157:H7. In field trials, applied *E. coli* were detected for less than five to 28 days. Recovery corresponded with irrigation volume; for example:

3.2 log CFU/g and 1.6 log CFU/g dw of soil for 10.7 cm and 7.1 cm of cumulative applied water, respectively. PFGE analysis revealed that two strains comprised more than 80% of colonies. *E. coli* were not recovered from any plant sample. *E. coli* were detectable in run-off for at least 5 weeks; transference to plants was not detectable.

Significance: These field trials demonstrate the challenges in predicting persistence and transference from soil to lettuce under commercial conditions. A simulated, single contamination event would be expected to have a short duration of persistence and very low level of transfer potential.

T4-10 Growth of *Escherichia coli* O157:H7 on Commercially Packaged Fresh-Cut Salads

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Introduction: An increasing number of foodborne illness outbreaks have been associated with the consumption of packaged fresh-cut lettuce and baby spinach contaminated with *E. coli* O157:H7. Since fresh produce grows in open fields, pathogen contamination can occur anywhere from farm to table. Moreover, fresh-cut products are marketed as Ready-to-Eat without a further microbial killing step. Therefore, preventing pathogen contamination and limiting its growth are critical measures to ensure fresh-cut produce safety.

Purpose: The main objective of this study was to understand the ecology of artificially inoculated *E. coli* O157:H7 on commercially packaged fresh-cut lettuce and baby spinach and to investigate the effect of storage temperature on the growth of *E. coli* O157:H7 and other background microorganisms as well as on product quality.

Methods: Recently delivered commercially packaged fresh-cut lettuce salad and baby spinach were obtained from retail stores. The packages were cut at the four corners and sprayed with *E. coli* O157:H7 inoculum. The packages were re-sealed and stored at 5 and 12°C until the expiration of their marked shelf life. *E. coli* O157:H7 and various background microorganisms were enumerated on selective media and product quality was evaluated by a trained panel. Data were analyzed with the General Linear Model Procedure, using SAS.

Results: Significant growth of *E. coli* O157:H7 was noted on packaged lettuce salad stored at 12°C, in which the population increased by more than 2 log CFU/g within 3 days. Aerobic mesophillic bacteria, yeast and mold populations also increased significantly while the visual quality of the product remained fully acceptable. No growth of *E. coli* O157:H7 was noted on packaged products held at 5°C throughout the entire storage period.

Significance: These data suggest that *E. coli* O157:H7 can grow significantly on commercially packaged fresh-cut products when temperature abuse occurs in the supply chain. Maintaining fresh-cut products at 5°C or below can help mitigate food safety risks if pathogen contamination does occur.

T4-11 DSC Glo Germ as a Cross-Contamination Indicator during Processing of Leafy Greens

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Introduction: Glo Germ, a fluorescent powder originally developed to assess hand-washing efficacy, has also been used to identify food contact surfaces on delicatessen slicers and aerosol production during mail sorting in response to bioterrorist concerns involving anthrax spores.

Purpose: This study investigated the ability of Glo Germ powder to identify and quantify the extent of contact between leafy greens and equipment during commercial processing.

Methods: Triplicate batches of retail baby spinach and shredded iceberg lettuce (22.7 kg) were surface-inoculated by immersion in 75 L of a 0.35% aqueous Glo Germ suspension. After 18–24 h of draining/drying at 4°C, both products were processed using a commercial flume tank, shaker table and a dewatering centrifuge capable of processing about 8500 kg of leafy greens/h. Immediately after processing, 23 product contact surfaces (10×10 cm^2) on the flume tank (11), shaker table (9) and centrifuge (3) previously identified by black light were swabbed, using EtOH-moistened Kimwipes, homogenized in 10 ml of EtOH, and then spectrophotometrically analyzed at 365 nm to determine the extent of product contact.

Results: Spinach and shredded lettuce respectively yielded OD values of 0.916 and 0.416, and 0.202 and 0.053, before and after processing. OD values during spinach processing ranged from 0.036 to 0.178 for the flume tank, 0.043 to 0.121 for the shaker table, and 0.071 to 0.200 for the dewatering centrifuge. The flume tank center, fume tank exit, shaker table entrance and centrifuge drain yielded significantly higher ($P < 0.05$) OD values of 0.118, 0.178, 0.121 and 0.200, respectively. Similar results were generally obtained for lettuce.

Significance: This is the first report showing that Glo Germ can be used to identify and quantify the extent of contact between foods and contact surfaces, with these findings also likely to be useful for identifying microbial harborage sites on equipment.

T4-12 Evaluation of the Sensitivity and Specificity of Rapid Test Kits for Detection of Pathogenic *Escherichia coli* O157:H7 from Lettuce and Leafy Greens

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Introduction: Multistate outbreaks of *E. coli* O157:H7 in 2005–2006 on fresh and especially fresh-cut produce greatly escalated the application of rapid pathogen detection systems to safety management in this food category. In particular, immuno-diagnostic and qRT-PCR platforms were rapidly integrated into Test and Release monitoring to qualify fields prior to harvest, incoming produce, and finished product.

Purpose: To enhance consumer safety and provide guidance for the industry, an assessment of the specificity and sensitivity of common kits was undertaken.

Methods: Five different methods were compared; Neogen's Reveal, SDI's RapidChek, Biocontrol's GDS, Qualicon's BAX and a regulatory method that uses

Matrix's PATHATRIX IMS system, viable recovery, and PCR. Nine types of leafy greens were freshly harvested and inoculated with approximately 10 CFU/25 g sample of a five isolate mixture of *E. coli* O157:H7 and then enriched following the recommended protocol (media, temperature, time, processing).

Results: At 8 h, for GDS, BAX, Reveal and Rapid-Chek respectively, there were 4, 6, 30 and 58% false negatives. False negatives for RapidChek were reduced to 16% when samples were enriched for an additional four hours. Platforms based on PCR as the initial confirmatory detection reaction were most consistent in identifying the presence or absence of the inoculated pathogen in the shortest period of time. Lateral flow immunoassays were not as sensitive. For red pigmented leafy vegetables, curly endive, red butter and lollo rosa, there were 38, 88 and 100% false negatives observed with the immunoassay tests while PCR detection was unaffected. Additionally, lateral flow systems were negatively affected by delays in achieving critical threshold populations during enrichment. No issues of specificity, false positives, were observed under test conditions.

Significance: Findings strongly suggest that product testing systems using 6–12 h detection cutoffs, currently reported by industry, result in false negative outcomes. These facts become very important in high-throughput testing and retest policies for presumptive positive lots.

T5-01 DSC Comparative Evaluation of Stomacher®, Pulsifier®, Bagmixer®, and Smasher® for Sample Preparations of Foods for Viable Cell Count and Coliform Counts

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Introduction: Microbiological examination of solid and liquid foods usually involves putting a sample (25 g or 25 ml) in 225 ml of sterile diluent (0.1% peptone water) in a sterile bag to make a 1:10 dilution. The mixture is then treated mechanically (shaking, massaging, vibrating) by an instrument to homogenize the food before plating for microbial counts.

Purpose: This research aimed to evaluate the Pulsifier®, Stomacher®, BagMixer®, and Smasher® with regard to Viable Cell Counts and Coliform counts of several types of food products as well as the noise level of each machine.

Methods: This report describes the effectiveness of Pulsifier®, Stomacher®, BagMixer®, and Smasher® in obtaining: (1) Viable Cell Counts and Coliform counts of spinach, nuts, ground beef, fish, alfalfa, sprouts, hot dogs, tofu, chicken wings, and chicken drumsticks, (2) noise levels as assessed by four people (noisy, acceptable, or quiet) and Decibel Meter (reported as Db, Speurders Co.) at five feet from each instrument during food preparation procedures, and 3) ease of cleaning of the instruments after use. ISO Method 7218:2007 was used for Total Viable Cells and Coliform Counts.

Results: Results indicated that the nine foods had different levels of microbes, but for individual food types, the counts were within one log of each other as obtained from the four instruments. Noise Level (noisy, acceptable, or quiet) and Decibels in descending order was Pulsifier® (77.11Db), Stomacher® (68.67Db), BagMixer® (66.44Db), and Smasher® (63.11Db). Ease of cleaning in descending order was: Stomacher®, BagMixer®, Pulsifier®, and Smasher®. Except for one sample, all the Db values of all instruments and food types treatments were below the OSHA standard of noise at 80 Db.

Significance: This research indicates that all sample preparation machines were comparable in their ability to recover microorganisms. While all machines were below the OSHA standard of noise, the Smasher® was found to be the quietest.

T5-02 Collaborative Study to Evaluate a Total Bacteria Count Assay Using Quantitative Real-Time PCR

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Introduction: A collaborative study was undertaken to demonstrate the precision and accuracy of a total bacteria count (TBC) assay developed to run with commercially available PCR reagents and a rapid thermocycling platform. The TBC assay can be completed within one hour. The TBC assay has many applications for enhanced HACCP applications.

Purpose: This study was designed to show how diverse laboratories can use the TBC assay in their labs for specific applications. Once a standard curve is constructed for a sample type, it can be used repeatedly for quantifying unknown samples.

Methods: A collaborator kit was supplied to each lab with all the necessary reagents and consumables that were detailed on a chain of custody document. Collaborating laboratories were:

Brigham Young University
California Department of Public Health
NASA Jet Propulsion Laboratory
NASA Ames Research Laboratory
Nestle USA Quality Assurance Center
North Carolina State University
San Francisco Water Quality Division

Results: Results from the 7 collaborative labs were analyzed using ANOVA and Signal to Noise ratio statistics (Taguchi Method) and showed excellent results for the standard curve and some variation in the unknown data. The β value indicated how close the average result was to the target and ranged from 0.87 to 1.07 (1.00 target). The signal to noise ratio (S/R), which includes the variance and β factor as the indicator of method whole performance, ranged from 14.22 to 23.18 (20.00 target).

Significance: The overall conclusions are that all the labs performed well in constructing the standard curve and had some variance with the unknown samples. However, the results show that all collaborators successfully used the TBC assay to quantify bacterial load accurately within one hour. The TBC assay can be used for a rapid real-time measure of bacteria in many diverse applications and support HACCP for real-time statistical process control.

T5-03 Construction of an Internal Amplification Control by In Vitro Transcription for *Salmonella* Detection Using Real-Time RT-PCR

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Introduction: Internal amplification controls that safeguard against the possibility of false-negatives are crucial in reverse-transcriptase polymerase chain reactions (RT-PCR). This is important for pathogen detection from complex food matrices when PCR

inhibitors are present. An RNA internal amplification control (IAC) was developed for monitoring real-time-RT-PCR *Salmonella* assays based on in vitro transcription of PCR products, using a T7 promoter.

Purpose: To eliminate false negatives and increase assay robustness for *Salmonella* detection, an RNA internal amplification control (IAC) with a different size and Tm from the target InvA product was used in real-time RT-PCR with a previously described invA primer set.

Methods: Novel *stx*1 primers were designed using the Beacon Designer 4.0 software to amplify the *stx*1 region of *E. coli* O157:H7 DNA (109 bp). A primer set that contained the invA-*stx*1 forward and invA-*stx*1 reverse primers was constructed to obtain an invA-*stx*1 PCR chimer product (154 bp) using the 109 bp as template. A T7 promoter was added to the invA-*stx*1 forward primer and along with the invA-*stx*1 reverse primer yielded a 182 bp PCR product. In vitro transcription of this 182 bp product produced the RNA IAC. Various concentrations of IAC were used for optimization in the real-time RT-PCR assay for *Salmonella* detection with invA primers.

Results: Real-time RT-PCR detection of *Salmonella* Typhimurium was achieved up to 10^3–10^2 CFU/ml using IAC at an optimal concentration of 1.9 fg/ul. The RNA IAC (154 bp and Tm of 82°C) was clearly distinguishable from target *Salmonella* products (347 bp and Tm of 87.5°C). Cut-off was based on Cts using IAC with controls.

Significance: The application of IAC in RT-PCR assays allows one to safeguard against false-negatives due to presence of inhibitors and/or failure of reverse-transcriptase or DNA polymerase. This method has tremendous application potential for rapid and sensitive detection of *Salmonella* in fresh produce such as spinach, tomatoes and lettuce.

T5-04 Eliminating Sample Preparation for Real-Time PCR Food Pathogen Detection

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Introduction: Food samples and samples collected from the environment have high levels of inhibitors that affect PCR performance. Elaborate sample preparation and nucleic acid extraction methods have to be employed to remove inhibitors present in these matrices. We have developed a real-time PCR Environmental Master Mix that is resistant to relatively high levels of commonly found inhibitors.

Purpose: To simplify real-time PCR applications in food and environmental testing by developing a real-time PCR Master Mix that is resistant to relatively high levels of common inhibitors, thereby eliminating the need for sample preparation.

Methods: Real-time PCR was run on the Applied Biosystems 7500 instrument using Applied Biosystems *E. coli* O157:H7 or *Salmonella enterica* TaqMan® Detection kits. PCR inhibitors (hematin, humic acid or urban dust) were spiked directly into PCR reaction mixes. Food samples were spiked with 3 CFU of pathogen and enriched overnight by standard methods (25 grams food in 225 ml broth).

Results: The environmental master mix detected 2 genomic equivalents of *Salmonella enterica* in the presence of 20 uM hematin, 15 ng humic acid, or 1.4 µg urban dust. Under the same conditions, another master mix showed no detection of *Salmonella* even at 2000 genomic equivalents. Enriched food samples spiked with *E. coli* O157:H7 or *Salmonella enterica* were tested for presence of pathogen by real-time PCR using the environmental master mix (without sample prep), and a confirmatory test (with sample prep). The results showed 100% correlation between the two methods for ground beef, cheddar cheese, mayonnaise, spinach, chocolate, and apple juice. Only raw milk was not detected at 100% (the master mix detected 6 of 11 confirmed positive samples).

Significance: Eliminating sample preparation for PCR applications simplifies food and environmental testing. Significant advantages include faster time to result, less chance for operator error, and reduced costs.

T5-05 DSC Evaluation of Fecal DNA Purification Methods and Conventional Culture Methods for the Detection of *Escherichia coli* O157:H7 in Feces of Naturally Infected Feedlot Cattle

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Introduction: Several protocols have been used on human and animal feces to purify DNA for PCR: direct DNA extraction from feces, DNA extraction with previous enrichment in selective media, and the use of commercial kits that involve the use of spin columns. The presumptive identification of *Escherichia coli* O157:H7 is possible in an individual, nonmultiplexed PCR if the reaction targets the gene of interest.

Purpose: The objective of this study was to compare the accuracy of the QIAamp DNA Stool test in detecting *E. coli* O157:H7 in enrichment and non-enrichment cattle feces from naturally infected feedlot cattle.

Methods: With use of conventional culture methods (gold standard), 456 fecal samples were analyzed for detectable levels of *E. coli* O157:H7. QIAamp DNA Stool Kit was used to purify fecal DNA (from enrichment and non-enrichment feces) and *E. coli* O157:H7 genes (*Stx*1 and *Stx* 2) were targated and amplified by PCR.

Results: 199/456 (43.6%) fecal samples were positive for *E. coli* O157:H7 by culture. Of the 456 enrichment fecal samples, *E. coli* O157:H7 Shiga toxin-like genes were detected from 159/456 (34.6%) enrichment fecal samples compared to 43/456 (9.4%) targated from non-enrichment samples. There was a substantial agreement (Kappa > 0.61) between the gold standard and QIAamp DNA Stool Kit (enrichment) as opposed to a fair agreement (Kappa > 0.21) for non-enrichment samples. Positive agreement rates were greater for the enrichment samples (83.6%) when compared with the non-enrichment samples (65%). Agreement rates were lower (86.4%) for QIAamp DNA Stool Kit for enrichment samples compared to non-enrichment samples (98.5%).

Significance: The use of the QIAamp DNA faecal purification kit provided rapid *stx* gene detection by PCR in cattle feces. However, the accuracy of detection was 83.6% and 65% for the enrichment and non-enrichment fecal samples respectively.This needs to be factored in the choice of protocols for *E. coli* O157:H7 detection in cattle feces.

T5-06 DSC Evaluation of an Automated ELISA and Real-Time PCR by Comparing with Conventional Culture Method for the Detection of *Escherichia coli* O157:H7 in Selected Foods

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Introduction: *Escherichia coli* O157:H7 is an important emerging foodborne pathogen that has become a constant threat to public health. Hamburger and radish sprout contaminated with *E. coli* O157:H7 have been implicated in serious outbreaks.

Purpose: The purpose of this study was to evaluate two rapid detection assays, VIDAS® (bioMérieux) and ABI7500 real-time PCR® by comparing them with a culture method for detection of *E. coli* O157:H7 in artificially inoculated ground beef and radish sprout.

Methods: The food samples (500 g) were artificially inoculated with nalidixic acid resistant *E. coli* O157:H7 strain. Inoculation levels were ≤ 0.04 CFU/g in ground beef and ≤ 2.8 CFU/g in radish sprout. The inoculated food samples were then divided into 20 samples (25 g each) to generate partial positive samples. All of the inoculated and uninoculated control samples were analyzed by culture method on CT-SMAC, VIDAS™ and real-time PCR. Nal-SMAC was used as selective agar for the gold standard. The real-time PCR assay was performed by use of a commercial kit that detects stx_1 and stx_2 gene.

Results: The level of inoculum to generate partial positive samples out of all samples tested were higher in radish sprout samples than in ground beef due to the high number of background flora. In ground beef, the number of positive samples detected by culture Method 65% (13/20) was similar with VIDAS™ 75% (15/20) and real-time PCR 70%, (14/20) while the gold standard method gave 75% (15/20) positive samples. In radish sprout, the numbers of positive samples were 16/20 (80%) with VIDAS™ and 13/20 (65%) with real-time PCR that were significantly higher than the culture method 35%, (7/20) while the gold standard showed the highest number of positive samples 90% (18/20).

Significance: It appears that higher selectivity of the culture media is required to detect *E. coli* O157:H7 in samples with higher background flora such as radish sprout. The results suggest that automated ELISA and real-time PCR could be useful alternative tools to the culture method for rapid and sensitive detection of *E. coli* O157:H7.

T5-07 DSC Evaluation of the Validity of Rapid Methods for Detection of *Listeria monocytogenes* in Various Food Samples

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Introduction: *Listeria monocytogenes* is an emerging foodborne pathogen and induces fatal listeriosis in pregnant women, infants, and immunocompromised people. Various detection methods have been used for prompt screening of *L. monocytogenes* from contaminated foods. Therefore validation of rapid detection methods is required in public health.

Purpose: The purpose of this study was to compare sensitivity of KOBAM methods (Korean Food Code Bacterial Manual) for the effectiveness of the incubation time suggested and to validate rapid detection methods, VIDAS™ (bioMérieux), and Reveal kit™ (Neogen), for *L. monocytogenes*.

Methods: *L. monocytogenes* was inoculated into various food samples (low level of background flora food—tofu, high level of background flora food—salads) to generate partial positive samples. The inoculated samples were pre-enriched in 225 ml of half fraser for 48 h (30℃). The enriched samples were streaked on Oxford Agar at 24 and 48 h and then two black colonies were transferred to TSA. Final identification was performed by Microgen TM *Listeria*-ID. To evaluate the validity of rapid detection method, VIDAS™ and Reveal kit™ were also performed using the 48 h-enrichment broth samples and compared with the culture method.

Results: Salad samples with high level of background flora required higher inoculum levels to generate partial positive samples out of the total of 20 samples tested in each experiment. When the enrichment period was extended from 24 (current KFDA method) to 48 h (USFDA method), the number of positive samples was increased from 0 (0%) to 4 (20%) in tofu and 4 (20%) to 16 (80%) in salad. There was no significant statistical difference between KOBAM and VIDAS™, Reveal kit™ in both samples.

Significance: It appears the current KFDA method (24 h enrichment) is insufficient to detect *L. monocytogenes*. Therefore the enrichment period should be extended to 48 h. Rapid detection methods (VIDAS™, Reveal kit™) could be adequate means for detection of *L. monocytogenes* in contaminated food.

T5-08 Performance of Media for Recovery of *Salmonella* from Thermally-Treated Egg White

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Introduction: Heat-injured *Salmonella* are often difficult to recover from liquid egg white (LEW). Numerous plating media have reportedly been used to recover injured *Salmonella*.

Purpose: This study was conducted to determine the performance of plating media for resuscitation and colony development by heat-stressed cells of *Salmonella* from LEW.

Methods: A six-strain composite of *Salmonella* was added to LEW (pH 9.0) at a population of 7.74 log CFU/ml, heated at 53.3°C for 3.1 min to induce ca. 2 log CFU/ml inactivation, and plated on twenty-seven non-selective and twenty-two selective media. Media were prepared according to manufacturers' directions, or prepared and supplemented with either NaCl, $KMnO_4$, EDTA, sodium deoxycholate, or calcium hypochlorite, and enrichment broths were solidified with 1.5% agar.

Results: Variations in ORP (80 – 263 mV) and pH (5.34 – 8.37) did not affect cell recovery. More cells recovered ($P < 0.05$) on five non-selective media (Tryptic Soy Agar [TSA] plus 0.01 or 0.1% EDTA, Difco plate count agar [PCA], TSA, and dextrose Tryptone Agar) than recovered on Brain Heart Infusion Agar, PCA (Oxoid), PCA (Sigma), Davis Minimal Agar, or on the following solidified broths: Luria Bertani, TSB, buffered peptone water no. 1 and no. 2, DE neutralizing, or brain heart infusion. Slightly greater numbers of

cells recovered on TSA with EDTA than on TSA alone, possibly due to chelation of hydroxyl-generating metals. More cells recovered ($P < 0.05$) on several selective media (brilliant green, Levine eosin methylene blue, brilliant green with phosphates, bismuth sulfite, and TSA with 1.5% NaCl) than recovered on XLD, XLT4, TSA with 3% NaCl, solidified selenite cystine broth, solidified Rappaport Vassiliadis broth, and solidified tetrathionate broth. Although $KMnO_4$ has been reported to enhance recovery of heat-injured *Salmonella*, those findings could not be replicated in this study.

Significance: The results reported here may assist in choosing plating media for recovering *Salmonella* from pasteurized LEW.

T5-09 Establishment of ELISA-LC/MS/MS System to Detect Aflatoxin B1 in Agricultural Products

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Introduction: In agricultural products, *Aspergillus* spp. have been known to produce aflatoxins, which are mutagenic and carcinogenic to humans. Thus, development of an efficient detection method is necessary for detection of the mycotoxin.

Purpose: This study established a detection method by using ELISA (enzyme linked immunosorbent assay) and high performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) for quantification of aflatoxin B1 (AFB1) in agricultural products.

Methods: A total of 387 samples (grains, nuts, beans, bean-paste, red pepper, red pepper paste) were purchased from six local retailers in six cities. Pulverized samples (5 g) were extracted by methanol: water (6:4, v/v), and the extract was diluted in phosphate buffered saline. The diluent was then added in microtiter plates coated with monoclonal antibodies for screening presumptive AFB1 positive samples by ELISA. Presumptive positive results were reextracted by methanol:water (9:1, v/v), and this extract was cleaned up by immunoaffinity column for LC/MS/MS (column: ZORBAX SB-Aq; mobile phase: water-acetonitrile-methanol (6:2:3, v/v); flow-rate: 1 ml/min) to measure levels of AFB1 in presumptive positive samples. To estimate recovery of the detection procedures, peanut and walnut samples were spiked at 0.5 and 1 ng/g (ELISA), 10 ng/g (LC/MS/MS), and AFB1 were detected using the method described above.

Results: Detection limits of AFB1 were 0.05ng/g (ELISA) and 0.1–0.5 ng/g (LC/MS/MS), and accuracy (recoveries) were 98.5–119.1% (ELISA) and 70.8 – 135.3% (LC/MS/MS). From screening AFB1 using ELISA, 22 samples (5.69%; peanut: 11, pistachio: 2, walnut: 6, almond: 1, pepper powder: 1, pepper paste: (1) were AFB1 presumptive positive. Of the positive samples, four samples (Peanut: 2, pistachio: 1, pepper powder: (1) were confirmed as AFB1 positive at levels of 1.02–52.79 ng/g after LC/MS/MS analysis.

Significance: The detection method using ELISA combined with LC/MS/MS may be more accurate and cost-effective in detection of AFB1 in agricultural products, compared to traditional methods.

T5-10 Rapid Assay for Detecting *Cryptosporidium parvum* in Milk Using Piezoelectric-Excited Millimeter-Sized (PEMC) Cantilever Sensors

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Introduction: Detection of foodborne parasites such as *Cryptospordium parvum* oocysts involve sample filtration to concentrate the target oocysts, followed by identification of using labeled reagents. Alternatively, the genomic DNA is extracted from the oocysts followed by amplification using PCR for detection. Such methods are tedious and require trained laboratory personnel. In this paper we describe a biosensor approach which offers a simple and a rapid method for detecting the oocysts in milk in 15 minutes.

Purpose: The purpose is to establish feasibility and determine detection limit of cantilever sensors for detecting *Cryptosporidium parvum* oocysts (Cpo) in milk samples.

Methods: Plezoelectric-excited millimeter-sized cantilever (PEMC) sensors were fabricated and immobilized with goat polyclonal antibody (Ab) specific to Cpo. Milk samples spiked with Cpo at 10–1,000 oocysts/ml were contacted with Ab-immobilized PEMC sensors and the resulting sensor response was recorded. Control experiments with Cpo-free milk and phosphate buffered saline did not induce a response. Confirmation of sensing was made using two methods: (1) sensor response when the bound Cpo was released using a low pH buffer, and (2) sensor response to exposing the sensor to IgM antibody against Cpo after the sensing episode.

Results: The PEMC sensor's resonant frequency decreases when target Cpo binds to it. The total resonant frequency change for the samples 10–1,000 per ml gave progressively increasing sensor response. Specifically, one ml of sample containing 10 Cpo gave a response of 200 Hz while a concentration of 5 Cpo/ml of 30 ml sample in a once-through mode gave a response of 520 Hz. Positive detection of Cpo in the sample solution was observed within 15 minutes. Positive (Cpo present, no Ab on sensor), negative (Cpo absent, Ab on sensor), and buffer (Cpo absent, no Ab on sensor) controls gave zero sensor response (~12 Hz). Verification of Cpo attachment was confirmed by low-pH buffer (pH 2.2) release, microscopy analysis, and second antibody binding post- Cpo binding. The results indicate that PEMC sensors can reliably detect *Cryptosporidium parvum* oocysts at 5 oocysts/ml in 15 minutes without sample preparation and without the use of label-free reagents.

Significance: The 15-minute TTR method developed with milk samples in this study is suitable for detecting any analyte in food matrices with the appropriate antibody immobilized on the sensor.

T5-11 The Use of Propridium Monoazide (PMA) to Distinguish between Viable and Dead *Clostridium sporogenes* (PA 3679) Spores after Thermal Processing

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Introduction: DNA can persist in the environment long after cell death, making it difficult to accurately quantify total viable bacteria using quantitative real-time PCR (qPCR). Recently, propidium monoazide (PMA) has been used to selectively distinguish between viable and dead bacterial cells since the dye can penetrate the

membrane of only dead cells. Both dyes intercalate with the DNA and irreversibly photo-crosslink, making the DNA unavailable for PCR amplification.

Purpose: The purpose of this research was to develop a rapid method to detect and quantify viable spores of *Clostridium sporogenes* (PA 3679), a commonly used *C. botulinum* surrogate, after thermal processing.

Methods: Spores were inactivated by heat treatment and the outer spore coats removed in a urea-based extraction buffer. PMA was added at a concentration of 12.5 µg/ml to the spores and crosslinked to the DNA with a 500 watt light. Total viable and dead spores were quantified using a SYBR green-based qPCR.

Results: The detection sensitivity for the qPCR assay as applied to serially diluted spores and spore-derived DNA was approximately 10^2 and 10^3 per reaction, respectively. PMA did not affect the recovery or amplification of DNA from viable spores. In addition, spore viability was not affected by removal of spore coats, as verified by loss of refractility under phase contrast microscopy, direct plating and correlation with qPCR results. Further studies showed that, even in the absence of the outer spore coat, the inner cortex layer still posed a considerable hurdle to complete incorporation of PMA to the interior of inactivated spores. However, a lysozyme treatment (50 µg/ml) prior to DNA extraction improved PMA incorporation, as seen by a shift in Ct values.

Significance: With further development, this is a promising molecular approach to evaluate thermal process efficacy, facilitating rapid enumeration of surviving *C. sporogenes*, an important *C. botulinum* surrogate.

T5-12 DSC Fourier Transform Infrared (FT-IR) Spectroscopic Methods for Analyzing Biofilms on Food Equipment Surfaces

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Introduction: Biofilms pose a significant concern in the food industry as sources of contamination with pathogenic and spoilage microorganisms. Their complete removal from processing equipment surfaces is highly desired to ensure food safety and quality. Thus, rapid methods for detecting biofilms will be very useful for the food processing industry to assess the efficiency of equipment cleaning procedures.

Purpose: In this study, the use of FT-IR spectroscopy was evaluated for the analysis of biofilms from stainless steel surfaces, using *Pseudomonas fluorescens* grown in milk as a model system.

Methods: Two methods were compared for analyzing biofilms: direct analysis on stainless steel coupons by FT-IR microspectroscopy and an indirect method involving biofilm recovery from surfaces prior to IR analysis and cell counting. Indirect analysis tested three commonly used solvents (phosphate buffered saline, PBS; distilled water, DW; and 0.85% NaCl) for maximum viable cell recovery from biofilms and least interference with IR spectral analysis.

Results: Direct analysis of biofilms was rapid and required minimal sample preparation. This method was suitable for analysis of lightly to moderately soiled surfaces. The indirect method allowed the examination of wider range of soil levels, enabling the detection of low amounts of biofilm on surfaces, as well as the analysis of heavy soils. The use of NaCl resulted in high viable cell recovery with minimal interference to biofilm IR spectra. Characteristic "fingerprint" spectral patterns showing compositional changes were observed in the biofilm during the different stages of its development.

Significance: Our results demonstrate the potential of FTIR technologies for providing information on the nature of contaminants present on equipment surfaces and the biochemical changes associated with biofilm formation. Successful development of these methods would enable the rapid detection and quantification of biofilms on food processing equipment and could serve as an important tool for monitoring the effectiveness of plant cleaning practices.

T6-01 DSC Registered Dieticians and Registered Nurses Lack Awareness and Knowledge of *Listeria monocytogenes*, Indicating Need for Continuing Education

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Introduction: Listeria monocytogenes is a foodborne pathogen that causes listeriosis, which can lead to septicemia, meningitis, or spontaneous abortion. Invasive listeriosis occurs primarily in individuals who are pregnant or who are immune compromised.

Purpose: Persons at risk for listeriosis should be informed of their risk and learn protective strategies. Because patients consider health professionals their primary source of food safety information, we conducted this research to assess registered dieticians' and registered nurses' knowledge of *L. monocytogenes*.

Methods: A web-based survey was designed for dietitians and nurses who currently provide service to pregnant women, elderly patients, and/or patients who are immune-compromised due to cancer, HIV/AIDS, or other disease. The survey included questions on knowledge of *Listeria monocytogenes*, the role of health professionals as providers of food safety information, their educational training needs, and demographic information.

Results: Sixty-one nurses and 73 dieticians completed the web-based survey. Nurses (98%, n = 60) said they have heard nothing or very little about *Listeria monocytogenes*, while 70% of dieticians (n = 51) had little awareness. Nurses (26%, n = 16) reported that they were not sure what foods were risky for *Listeria monocytogenes* contamination, while 8% of dieticians (n = 6) reported the same. Only 28% of nurses (n = 17), but 88% (n= 68) of dieticians, said that they have received specific training on food safety, while most of them showed interest in participating in an educational program on *Listeria monocytogenes* (95% and 100%, respectively).

Significance: These data suggest specific educational training for health professionals is justified.

T6-02 Best Practice – Occupational Exposure Control Plan for Restaurants and Food Establishments

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Introduction: Restaurant and processing organizations have expressed concern, primarily from a Risk Management perspective, if in fact an Occupational Exposure Control Plan (Bloodborne Pathogen Program) is needed or required within their eating establishments. Additionally, recent changes in the US Food Code related to inclusion of the highly transmissible foodborne

pathogen Norovirus raises concerns on proper control programs for risk management. Norovirus is the fastest emerging reported foodborne illness within the US.

Purpose: Typically, many restaurants and manufacturers, especially smaller food processors, are ill equipped to handle and remediate a body fluid spill of any kind in the event of an exposure incident. This Best Practice will provide guidelines for organizations to format a plan of action.

Methods: The conclusion drawn from a variety of facts and the author's research has led to the conclusion that the restaurant industry is very vulnerable, with significant risk for potential exposure. It is therefore highly recommended to have in place an Occupational Exposure Control Plan, compliant with the US Bloodborne Pathogens Standard or Universal Precautions for the restaurant and food establishment Industry. This plan if properly implemented would also sufficiently manage concerns with transmissible foodborne pathogen clean-up and control as well.

Results: Aside from a potential Occupational Exposure risk, within the restaurant or foodservice industry the added danger of a potential foodborne illness contamination also exists. A body fluid spill incident, such as a vomitus episode, could pose a serious foodborne illness crisis situation if not properly contained and cleaned. The format suggested is a Best Practice approach to remedy such a potential exposure incident.

Significance: The organization, its brand, employees and consumers must come first in examining any risk scenario. A smart risk minimization platform of having an Occupational Exposure Control Plan in place within food establishments far outweighs any potential exposure liability occurrence.

T6-03 DSC An Observational Study of Food Safety Practices at Food Service Conducted through Video Capture

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Introduction: It is estimated that food service can be associated with 40–70% of foodborne illness outbreaks. Past research into the food handling practices at food service operation have been limited to self-reported data or researcher-present observational studies.

Purpose: This research, which was undertaken to capture and categorize food safety practices of food service front-line staff, used a unique video recording technique. This exploratory research was intended to provide a baseline of practices and profiles of food handlers to be used in risk-reduction program development.

Methods: Through a major international food service company, a convenience sample of 10 sites was used for data collection. Between four and 16 surveillance cameras were secured within food handling and preparation areas at each site to capture food handlers' practices from different viewing angles. Both quantitative (tabulation of observed preparation practices) and qualitative (attitudes and intentions surrounding safe food preparation acquired through in-depth interviews) methods were used.

Results: Food preparation was completed by staff who did not always practice foodborne illness risk-reduction procedures. Practices viewed that could contribute to foodborne illness included improper cooking procedures; lack of hygiene and sanitation by food handlers; and cross contamination between raw and fresh, ready-to-eat foods. Profile characteristics of observed participants (including age, experience, formal food safety training and understanding of microbial food safety hazards) contributed to the frequency of improper practices.

Significance: Often a focus on efficiency of meal preparation takes priority, in spite of efforts to address microbial food safety with food handlers. Data collected through direct observation studies more accurately reflects actual food handling practices than results of self-reported surveys. Categorization and tabulation of practices can also be used in future risk assessments.

T6-04 Transportation Risk Assessment for Food Safety and Security; An Examination of Risks and Solutions Associated with Food Transportation

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Introduction: The food we eat travels on average 1,300 miles domestically, 30 billion tons of food arrive at 132 United States ports each year, and 90 percent of the US food supply is transported by truck. This research was conducted to develop an understanding of the risks to our food supply, and possible solutions to those risks, of transporting food by truck. Cooperation, communication, and dedication by four states and five agencies made this research possible.

Purpose: This project sought to determine the current state of food safety and defense of in-transit food in interstate commerce, and to identify and test mechanisms for effective coordination between multiple states and agencies.

Methods: Drivers in four states were stopped. On-site interviews determined the trucking firm's transportations practices and driver's knowledge of food safety and defense. Truck shipments were also inspected in collaboration with public health and state police agencies in order to assess the risks to food safety and defense present during truck transportation.

Results: More than 25,000 trucks were observed and categorized in four states. Six hundred-fifteen (615) drivers participated in the written survey: Illinois, 111; Indiana, 117; Michigan, 168, and Ohio, 219. Enforcement, including dumping of cargo, was conducted by participating agencies when necessary. The research demonstrated that mixed loads with multiple destinations had the most inadequate product segregation. In addition, smaller box trucks and ethnic food transportation had the majority of safety and security issues, with more than 80 percent of all drivers lacking food safety and defense knowledge.

Significance: Current food transportation practices threaten our food supply safety and defense. Education for drivers is needed, along with multi-state, multi-agency coordination to increase the efficiency and effectiveness of interstate transportation food safety assessments.

T6-05 Consumer Attitudes to Food Safety in Mexico

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Introduction: Foodborne diseases and poisoning might come from poor food handling at home, such as cross contamination, inadequate food storage, cooking, reheating and poor hygiene. Thus, consumers are also responsible for ensuring food safety at home.

Purpose: The aim of the study was to determine attitudes, knowledge and awareness among consumers regarding food safety.

Methods: The questionnaire applied was adjusted on the basis of the experience gained in a previous pilot study based on personal interviews. A total of 110 university students received the questionnaire; 88.2% (53 male and 44 female) turned in questionnaires fully answered.

Results: Food safety was an issue of great concern to the majority and on-campus information, internet and books were pointed out as main sources of information. Origin of food products and food handling at supermarkets were indicated by 69% of the students surveyed as the two main ($P < 0.001$) factors to be associated with higher food safety risks. In markets of rural areas, 80% of the students surveyed indicated that the major risk to food safety was food handling. Reading of food labels was a common practice in 61% of the students surveyed.

Significance: As all of the students indicated a great concern of food safety issues, it was concluded that the topic of food safety should be considered a basic and essential life skill and a component of the college curriculum. Further studies will be necessary for an extensive description of Mexican consumers' attitudes toward food safety.

T6-06 Efficacy of Two Commercial Sanitizers and Two Conveyer Belting Systems against *Listeria monocytogenes* during Normal Operation

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Introduction: The food industry has historically faced many concerns regarding adequate design, cleaning and sanitizing of conveyor belts, with development of biofilms also hindering sanitizer efficacy.

Purpose: This study assessed the efficacy of two commercial sanitizers against *Listeria monocytogenes* (*Lm*) using a custom-built dual belt conveyor system designed for continuous/discontinuous inoculation and continuous spray sanitizing.

Methods: For conveyor belt inoculation, 15 liters of 10% turkey slurry containing a 6-strain *Lm* cocktail (10^7 CFU/ml) was placed in an inoculation tray. An Intralox (interlocking) and ThermoDrive (smooth) belt were then simultaneously passed through the inoculation tray for 3.5 min (5 revolutions, followed by tray removal) or 2 h for discontinuous and continuous inoculation, respectively. After initial quantification of *Lm* on both belts, two organic acid sanitizers — Octave (2340 ppm) and Vortexx (2294 ppm), XY-12 (180 ppm, chorine) and water (control) — were continuously sprayed onto both belts (30 L/h) during 2 or 3 h of operation. At pre-determined intervals, two interlocking belt segments were removed and sonicated in neutralizing buffer for 10 min, whereas the ThermoDrive belt was sampled using 1-ply composite tissues. Appropriate dilutions were plated on Modified Oxford Agar overlayed with a non-selective medium to quantify *Lm*, with membrane filtration used when low populations were expected.

Results: Using discontinuous inoculation, Vortexx and Octave decreased Lm populations > 5 and > 4 logs ($P < 0.05$) on the ThermoDrive and Intralox belts after 15 and 20 min respectively, while XY-12 and water were significantly less effective. In contrast, *Listeria* populations decreased < 3 logs during 2 h continuous inoculation with no significant difference seen between Vortexx, Octave and XY-12. Regardless of the inoculation method, significantly greater reductions were seen using the ThermoDrive belt.

Significance: Both Vortexx and Octave were effective sanitizers for these conveyor belts with the ThermoDrive belt offering greater cleanability.

T6-07 DSC Beyond Intent — Direct Observation of Meal Preparation Procedures in a Home Kitchen Setting

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Introduction: There is a lack of research examining whether safe food handling labels are perceived as effective and translate into actual safe food handling behavior, including the use of proper thawing and cooking techniques, the use of measures to minimize cross contamination, and the use of meat thermometers to confirm doneness.

Purpose: The purpose of this study was to use a novel video capture system to observe the kitchen preparation practices of 40 consumers — 20 primary meal preparers and 20 adolescents — using uncooked, frozen, breaded chicken products and to determine if differences exist between consumers' intent and actual safe food handling behavior.

Methods: A convenience sample was used for participant selection because participation was voluntary, and required that participants be video-recorded preparing food in one of two model kitchens at Kansas State University. The kitchens used were diagonal to one another and had different layouts but contained the same appliances and utensils. Three small surveillance cameras (QuickCam Pro 5000, Logitech) were strategically secured within each kitchen to capture the participant's preparation practices from different angles.

Results: There were significant differences between self-reported and actual observed food safety behaviors. A relatively high number of all participants, 73 percent, were observed washing their hands prior to beginning food preparation for the first time. On average, adolescents re-read the label instructions more times than the primary meal preparers. Adolescents also made significantly more food preparation mistakes compared to the primary meal preparers. Although many participants reported owning a food thermometer (73 percent) and using it when cooking raw breaded chicken entrees (19.5 percent), very few participants were observed measuring the final internal temperature with a food thermometer.

Significance: Data collected through direct observation more accurately reflects actual food handling behaviors than data collected through self-reported surveys.

T6-08 Consumers and Take-Out Food: Safe-Handling Practices, Desired Packaging Attributes, and Temperature Integrity of Packaging

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Introduction: Consumers' purchase of food-away-from-home increases annually, with convenience products now responsible for up to 10% of total sales. This can provide a huge amount of profit for chains since 57% of the population orders take-out food at least once per week. But with this arises concern as to the public's knowledge of safe food handling.

Purpose: To determine awareness of food safety issues associated with food prepared away-from-home, attributes of take-out packaging consumers want, and to determine the temperature integrity of widely used take-out containers using on-site field testing.

Methods: Online survey used covering topics as sanitation practices and how food is treated once purchased. A 5-point Likert scale and multiple choice questions were employed. Cytherm datalogger keys were used to track temperature of food purchased three ways; entered establishment and ordered food, ordered food from drive-through, and called in orders/car-side. Key was inserted into product and package resealed. Key remained in package for two hours at room temperature.

Results: Sample bought take-out an average of 6.84 times/month spending an average of $80.53. Packaging characteristics most wanted; leak proof (95%), keep food hot/cold (91.9%), keep food from becoming soggy (83.8%). Type of food safety information provided; none (82.2%), reheating instructions (22.2%). Sample agreed they: understood how to handle leftovers (57%), returned home as soon as possible (54%), understood what causes foodborne illness (52%), and were knowledgeable in food safety (52%). Packaging that did not keep food hot; microwavable, corrugated and paperboard boxes, aluminum foil, and plastic clamshell. Microwavable containers lost an average of 15% of temperature in 30 minutes. Packaging that performed well; polystyrene, some foil wrappers, and waxed containers. Polystyrene lost an average of only 4% of temperature in first 30 minutes. Most food was in the Temperature Danger Zone when purchased.

Significance: Study showed consumers were somewhat knowledgeable in food safety, but not enough to handle the food once it left the establishment, so restaurants would be wise to provide food safety information to their consumers, especially since most food was in the temperature danger zone when purchased. Study can also help restaurants decide the type of packaging and features to use according to what consumers want and the ability of the packaging to keep foods hot.

T6-09 DSC Validation of a Four-Chain Quaternary Ammonium Compound (A) and Polymeric Biocide (B) for Inhibition of *Listeria monocytogenes* Attachment on Food Contact Surfaces

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Introduction: The continuous presence of humidity and organic substrates make food processing environments and food contact surfaces very susceptible to microbial growth. Ineffective and inadequate cleaning and sanitizing procedures are some of the major causes of recontamination of food products with *Listeria monocytogenes.*

Purpose: The objective of this study is to validate the effectiveness of a Four-Chain Quaternary Ammonium Compound (A) and Polymeric Biocide (B) in reducing or inhibiting the colonization of *L. monocytogenes* populations on stainless steel. *Pseudomonas putida* was used in conjunction with *L. monocytogenes* as previous studies have shown this organism to aid in biofilm formation.

Methods: A five strain cocktail of *L. monocytogenes* and *P. putida* (5:1 ratio) was used to inoculate 24 polished stainless steel coupons (6.4 × 1.9 cm) divided into three treatment groups (8/set), of 400 ppm solution of (A), 400 ppm of (B), and DI water (control). Biofilms were allowed to develop for 12, 24, and 36 h. Populations of *L. monocytogenes* were calculated by dislodging attached cells by vortexing coupons for 1 minute with 20 g of 3 mm solid glass beads in 10 ml 0.1% peptone diluent and plating onto Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Agar Layer-MOX (TAL-MOX) Agar.

Results: L. monocytogenes was not recovered from either (A) or (B) treatments (D.L.= 0.9 $\log_{10}$ CFU/cm^2). From the control, the recovery rates were 2.9, 2.3, and 5.6 $\log_{10}$ CFU/cm^2 for MOX and 2.3, 3.4, and 5.3 $\log_{10}$ CFU/cm^2 for TAL-MOX, for 12, 24, and 36 h, respectively. Counts on TSA showed that *P. putida* attached to coupons with both the treatments, but the colonization rate remained below 3.5 $\log_{10}$ CFU/cm^2 for the 36 h incubation period.

Significance: This study indicates that these compounds tested are effective for use as cleaning and sanitizing agents on stainless steel for control of *L. monocytogenes* and reduction of biofilm formation.

T6-10 Presence of Aerobic Microorganisms, *Enterobacteriaceae* and *Salmonella* in the Shell Egg Processing Environment

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Introduction: Sanitation is vital to providing safe, healthful food to consumers. Understanding the degree to which microorganisms persist on specific equipment or locations contributes to developing effective sanitation programs.

Purpose: Certain microbial populations may be used to determine areas within a processing plant that warrant an increased effort to acheive an acceptable level of sanitation. A survey of aerobic microorganisms (APC), *Enterobacteriaceae* and *Salmonella* contamination of 26 sites within a single commercial shell egg processing plant was conducted on two visits.

Methods: Tank lids, nozzle guards, and the interior surface of the tank, spindles, brushes and belts along the processing chain, scoops, air filters, and floor drains were sampled, using swabs moistened with 10 ml deactivating buffer. Samples were transported on ice to the laboratory. Each sample was stomacher blended and then duplicate plated onto plate count agar, incubated for 48 h at 37°C and violet red bile glucose agar incubated overnight at 37°C to enumerate aerobic microorganisms and *Enterobacteriaceae*, respectively. Counts for each sample were converted to log CFU/ml and results from both visits were averaged. Sponges for each sample were selectively enriched, using conventional cultural media, incubation temperatures and times.

Results: APC levels ranged from 1.9 log CFU/ml for washer tank lid and post-wash spindles to 7.6 log CFU/ml for the pre-wash tank surface and floor drains. *Enterobacteriaceae* levels ranged from 0.0 log CFU/ml for wash tank nozzles, lids, post-wash spindles, and post-wash packer belt to 3.1 log CFU/ml for pre-washed egg accumulator belt, floor drains and 4.0 log CFU/ml for breaker egg diverter. *Salmonella* was recovered from floor drains, breaker egg diverter and breaker egg belt surfaces.

Significance: High levels of APC and *Enterobacteriaceae* may not always provide an index of pathogen contamination but they can be used to indicate locations within the processing environment where stricter hygiene is needed.

T6-11 Evaluation of Household Products as Sanitizers against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

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Introduction: Households need to have various means available to inactivate pathogens in their kitchens and to avoid creation of contamination niches, which may serve as sources of cross contamination of food.

Purpose: Selected household products were evaluated for their efficacy against foodborne pathogens.

Methods: Hydrogen peroxide (1.5 and 3%), undiluted (5%) acetic acid and 1:1 diluted vinegar, baking soda (11, 33 and 50% sodium bicarbonate) and household bleach (0.0314, 0.0933 and 0.670% sodium hypochlorite) solutions were tested for their effectiveness against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium individually (5-strain composites; 5×10^8 CFU/ml) by the AOAC International suspension test with some modifications at 25°C and 55°C for 1 and 10 min (two replications/three samples each).

Results: All bleach solutions (pH 8.36–10.14) produced > 5-log reductions of all pathogens tested after 1 min at 25°C, while all baking soda solutions (pH 7.32–7.55) reduced counts by < 1 log even after 10 min at 55°C. After 1 min at 25°C, 3% hydrogen peroxide (pH 2.75) achieved >5-log reductions in both *S.* Typhimurium and *E. coli* O157:H7, while undiluted vinegar (pH 2.58) and 1.5% hydrogen peroxide (pH 2.82) achieved > 5-log reductions only in *S.* Typhimurium. Compared to 1 min at 25°C, significantly ($P < 0.05$) higher reductions in *L. monocytogenes* were obtained with undiluted vinegar after 10 min at 25°C or 1 min at 55°C, with 3% hydrogen peroxide after 1 min at 55°C, and with 1.5% hydrogen peroxide and 1:1 diluted vinegar (pH 2.78) after 10 min at 55°C.

Significance: Sanitizing efficacy increased in the order: household bleach (0.0314%) > hydrogen peroxide (3%) > undiluted vinegar > baking soda (50% sodium bicarbonate). Based on the sensitivity sequence (*S.* Typhimurium > *E. coli* O157:H7 > *L. monocytogenes*), *L. monocytogenes* should be recommended as an indicator for testing sanitizer efficacy. The reaction temperature of 55°C and exposure time of 10 min enhanced sanitizer effectiveness.

T6-12 Food Safety Management in Fraser Health Authority, Vancouver, BC, Canada

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Introduction: In July 2000, the Fraser Health Authority (FHA) implemented new legislation that required risk-based inspection. Before this new legislation, public health inspectors would inspect foodservice facilities, to include restaurants, food processing plants and food stores, and find the same issues every inspection. The facilities were given inspection reports, and operators would correct the issues, but during following routine inspections, similar issues related to safe food handling, sanitation and maintenance were found, as with previous inspections. There are challenges with the new legislation, because many operators do not have English or French as their first language. They also have difficulty following the concept of food safety and sanitation procedures because of where they were raised and the fact that they have no restaurant operating experience.

Methods: To solve these problems, this new legislation requires food operations to attend mandatory classes taught by FHA. Also, they must submit food safety plans and sanitation plans before they can operate. In addition, operators must have at all times at least two staff with the FOODSAFE Level 1 course education. Inspections include review of food safety plans and sanitation procedures, and log sheets of daily monitoring of temperatures, and conducting a thorough building inspection to ensure premises are of sound structure.

Results: Implementation of this new legislation and mandatory self-control program has resulted in foodservice operators being responsible for their own operations and allows inspectors to focus on food safety procedures and sanitation procedures. The new risk-based routine inspections have resulted in significantly lower hazard ratings, as the operators take charge of their own operations.

Significance: This presentation discusses the improvements in food safety in the more than 7,000 food premises that make up the FHA. The presentation will also discuss how to overcome language barriers and cultural habits.

T7-01 Yeast and Mold Ecology in Food Factories

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Introduction: The ecology of food processing environments with respect to bacteria is now reasonably well established in terms of the factors that lead to environmental selection of bacterial types (e.g., psychrotrophs in chilled plants); and persistence and niche occupancy of factory strains and their growth. However, despite the fact that yeast and mold spoilage of some food products is a major concern, the ecology of factories with respect to these fungi is not so well understood.

Purpose: The purpose of this project was to determine whether the variety of yeasts and molds found in different food factories are selected for, whether they are persistent, whether they occupy

specific niches, and/or whether they are able to grow both in the environment and in products.

Methods: Ecological studies involving sampling of the factory environment and of spoiled product for yeast and mold isolates, have been undertaken at a commercial bakery, a dairy and a drinks factory over a two-year period. Selected product and environmental isolates have been visually characterized by genus and then analyzed on a molecular level using the Diversilab™ system. Current studies are focusing on the mold genus *Penicillium.*

Results: To date, more than 1,800 isolates have been collected. Molecular characterization using the Diversilab™ mold kit indicated that visually similar isolates from the product and environment may have different DNA fragment profiles. The technique has been able to discriminate between *Penicillium* spp. isolates, has proved useful for grouping genetically similar isolates (for matching of environmental and product isolates, and determination of persistence) and has provided some evidence of the same strains being found in the environment and the product.

Significance: The results of this work will be used to help provide a better understanding of the role of the factory environment in contributing to spoilage incidents and suggest appropriate factory based control strategies.

T7-02 Microbial Contamination during Production of Edible Peeled Chestnuts

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Introduction: Peeled chestnuts represent a growing value-added commodity in Michigan, with potentially expanded markets. Unlike most nuts, peeled chestnuts must be immediately frozen for later culinary applications.

Purpose: Concerns regarding the shelf-life and spoilage of peeled chestnuts after thawing prompted a 2006–2007 survey in which chestnuts were quantitatively examined for various microbial contaminants after harvest as well as during and after peeling.

Methods: Chestnuts were randomly collected after harvest from seven Michigan growers, stored at 4°C for 2 weeks, and peeled using a brulage peeler (Boema; Nelve, Italy). The processing line contained a chain batcher, peeling oven, tangential cleaner, elevator batcher, worm screw parboiler, skin separator and a sorting belt. Triplicate 25-g chestnut samples were diluted 1:5 in phosphate buffer solution (PBS), homogenized by stomaching, serially diluted in PBS and plated on trypticase soy agar plus 0.6% yeast extract containing cycloheximide for mesophilic aerobic bacteria (MAB) and potato dextrose agar containing streptomycin and ampicillin for yeasts (Y) and molds (M). Processing water (50 ml) and environmental swab samples (~10 × 10 cm) were also collected and similarly analyzed for MAB, Y and M.

Results: Average MAB, Y and M populations in chestnuts were 2.70, 2.74 and 2.51 at post-harvest; 3.46, 3.27 and 2.40 during peeling; and 5.39, 3.09 and <1.70 log CFU/g after peeling, respectively. Overall, microbial populations in the water and environmental samples increased from < 1.7 to 5.46 log CFU/ml and <1.7 to 5.91 CFU/10 cm^2, respectively. During processing, the skin separator and a sorting belt were identified as key points for contamination.

Significance: Since MAB and Y populations increased significantly ($P < 0.05$) by > 2 logs between harvest and after peeling, improved and more effective microbial reduction strategies after processing are needed to ensure the quality and prolong the shelf life of peeled chestnuts.

T7-03 Inactivation of Bacteria and Yeast on Peeled Chestnuts Using X-ray Radiation

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Introduction: An edible chestnut industry for fresh and peeled chestnuts has been established in Michigan. The shelf life for mechanically peeled, frozen and subsequently thawed chestnuts is only 13 rather than the required 28 days at 4°C because of microbial spoilage. In previous work, two bacteria — *Rahnella* sp. (R1) and *Curtobacterium* sp. (C1)— and one yeast — *Candida* sp. (Y1) — were the primary causes of spoilage.

Purpose: A newly developed low-energy X-ray food irradiator (Rayfresh Foods Inc., Ann Arbor, MI) was tested for extending the microbial shelf life of surface-inoculated chestnuts.

Methods: Peeled chestnuts (1500 g) were shaken (150 rpm) for 5 min in 4 liters of Lauria-Bertani broth containing 10^8 CFU/ml of R1, C1 and Y1 to obtain ~10^6 CFU/g on the chestnuts. After air-drying for 10 minutes, four 75-g chestnut samples (12 chestnuts/sample) were placed in a Whirl-Pak bag in a single layer and then surface-irradiated at doses of 0.5, 1.0, 1.5, and 2.0 kGy as determined by radiochromic film dosimetery. Immediately after treatment, and again after 10 and 18 days of storage at 4°C, the chestnuts (25 g) were added to 100 ml of phosphate buffer solution, homogenized by stomaching and then plated on Trypticase Soy Agar plus 0.6% yeast extract containing cycloheximide for enumerating R1 and C1 and on potato dextrose agar containing streptomycin and ampicillin for Y1.

Results: All doses significantly ($P < 0.05$) reduced Y1 by 1.30, 1.90, 2.11 and 2.56 log CFU/g and reduced both R1 and C1 by 0.34, 0.96, 1.07 and 1.58 log CFU/g, respectively, with all of these treatments significantly ($P < 0.05$) extending the shelf life from 13 to > 28 days compared to the non-irradiated controls.

Significance: Based on these findings, X-ray irradiation may provide an effective means of enhancing the microbial shelf life of peeled chestnuts.

T7-04 Multistate Outbreak of *Salmonella* I 4,[5],12:i:- Infections Associated with Commercially Produced Frozen Pot Pies — United States, 2007

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Introduction: Nationally, an estimated 45,600 *Salmonella* serotype I 4,[5],12:i:- infections occurred in 2006; yet risk factors for infection are poorly understood. In June 2007, PulseNet, the national foodborne infection molecular subtyping network, identified a multistate *Salmonella* I 4,[5],12:i:- outbreak.

Purpose: We investigated to determine the outbreak source and to prevent further illness.

Methods: We conducted a matched case-control study, defining a case as a *Salmonella* infection with the outbreak pulsed-field gel electrophoresis pattern in a

resident of a participating state ≥ 2 years old without ill contacts and onset during August 1–October 3, 2007. Case-patients were asked about exposures in the week before onset; age-group matched controls, selected via reverse directory, were asked about the week before interview. Focused questionnaires were requested from all patients with outbreak strain infections in 2007. Product was cultured for *Salmonella*.

Results: In 2007, 381 persons from 40 states had outbreak strain infections. Forty-eight patients met the study's case definition; 17 case-patients and 24 controls were enrolled. Illness was associated with eating Brand X frozen pot pies (matched OR 23.6, 95% CI 3.8–infinity). Brand X pot pie consumption was reported by 145 (72%) of 202 patients but no controls; 75% of patients cooked the pies in microwaves. Inconsistent microwaving instructions were printed on different parts of Brand X pot pie packages. Furthermore, although microwaving instructions varied by wattage, 70% of patients could not report their microwave's wattage. Nine Brand X pot pies yielded the outbreak strain.

Significance: Brand X pot pie microwaving instructions were confusing, and, even if followed accurately, were probably insufficient to consistently reach temperatures to kill *Salmonella*. All pot pies produced in the plant (~250 million) were recalled; subsequently, the manufacturer modified microwaving instructions. Attention is needed to decrease the risk of illness from frozen, processed, not ready-to-eat foods.

T7-05 Increasing Incidence of Listeriosis in France and Its Relations with Host Factors and Food Control

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Introduction: Listeriosis is caused by the bacterium *Listeria monocytogenes*, a foodborne pathogen. This disease, which is largely confined to risk groups including pregnant women, the elderly and immunocompromised individuals, has increased in incidence in France since 2006.

Purpose: Our objective is to describe this increase of incidence and investigates its origin, notably at the food control level.

Methods: Since 1998, the French surveillance system for human listeriosis relies on the mandatory notification of cases to the national public health institute (InVS) and the submission of *L. monocytogenes* strains to the national reference center (NRC, Institut Pasteur). The French surveillance system of food contamination by *L. monocytogenes* is based on the voluntary submission of strains isolated in the context of self-controls, or the mandatory submission to the NRC in case of official enquiries. These modalitles of collection of human and food data have been shown to be effective in controlling this foodborne disease.

Results: Based on surveillance data for 2006–2007, the French incidence of listeriosis had increased from 4.7 cases per million inhabitants in 2006 to an estimated value of 4.8 in 2007. The number of pregnancy-associated cases is stable, and this increase involves mostly sporadic cases of bacteremic patients over 60 years old. No outbreak explains this increase. The causes of this increase may be due to (1) the use of new immunosuppressive therapeutic regimens in patients over 60 years, (2) a change in food practices in this at-risk group, (3) a change in food industry practices such as food composition and/or shelf-life modification, and (4) a possible unforeseen side effect of the application of new European regulations on food hygiene.

Significance: In conclusion, in addition to the current efficient recommendations for prevention of listeriosis in pregnant women in France, new recommendations will have to be issued for individuals over 60 years, when the cause of this increase will have been precisely determined.

T7-06 Prevalence of Exposures to Raw Meat and Poultry Products among Children Riding in Shopping Carts: Increased Risk of *Salmonella* and *Campylobacter* Infection

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Introduction: Young children have the highest incidence of *Salmonella* and *Campylobacter* infection. While many infants do not consume foods typically associated with illness, a study of infants infected with *Salmonella* and *Campylobacter* found an association between illness and riding in a shopping cart next to raw meat or poultry.

Purpose: To better characterize this risk behavior, we surveyed parents of young children residing in Foodborne Disease Active Surveillance Network (FoodNet) sites.

Methods: A 12-month population-based telephone survey was conducted in 10 FoodNet sites in May 2006–2007. Respondents with a child < 3 years were queried regarding placement of children in shopping carts and their exposure to raw meat and poultry.

Results: Among 1,245 respondents with a child < 3 years, 953 (77%) reported the child being in a grocery store in the previous seven days. Of these, 829 (87%) rode in a shopping cart. Of 761 children who rode in the child seat and/or basket of the cart, 99 (13%) were exposed to raw products, 88 (12%) to meat and 73 (10%) to poultry. Children who sat in the basket (n = 142) were more likely than those who only sat in the seat (n = 611) to be exposed to raw product (OR 20.8; 95% C.I. 12.6–34.4). Compared to white non-Hispanic persons, persons of Hispanic ethnicity (OR = 4.4) and black non-Hispanics (OR = 4.0) were more likely to be exposed. Income, education and state were also associated with exposure. In a multivariate model, riding in the basket (OR 19.7; 95% C.I. 11.5–33.8) and income (OR 2.7; 95% C.I. 1.5–4.6) remained significant predictors.

Significance: Exposure to raw meat and poultry among children riding in shopping carts is not uncommon. To prevent transmission of *Salmonella* and *Campylobacter* infections, parents should restrict children from exposure to raw products by placing children and raw products in different areas of the shopping cart.

T7-07 Genotypic Similiarities and Antimicrobial Resistance Profiles of *Salmonella* Isolates in Humans and Animals in North Dakota

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Introduction: It is estimated that 95% of salmonellosis cases are foodborne. Epidemiological studies have established linkage between human salmonellosis and consumption of foods of animal origin or contact with live infected animals. As domestic animals are a primary source of human *Salmonella* infections, we hypothesized that *Salmonella* serotypes with similar genotypic profiles involved in human and animal outbreaks could have similar antimicrobial resistance (AMR) profiles with those causing parallel infection in domestic animals

Purpose: To investigate the similarities in the genotype and AMR profiles of the *Salmonella* isolates from humans and domestic animals outbreaks.

Methods: A Comparative Pulsed field gel electrophoresis (PFGE) study of 183 human and 230 domestic animals *Salmonella* isolates was used to demonstrate cross infection. Additionally, AMR profiles of isolates from human and domestic animals with 100% PFGE similarity were compared to determine AMR patterns and explain the role of *Salmonella* isolates from domestic animals as major source of resistance to human pathogens.

Results: A total 91 PFGE profiles (> 85%) were observed, of which 8 profiles had 100% similarity between 40 human and 51 animal isolates. Of the 26 antibiotic resistance profiles (phenotypes), only one human isolate shared the same resistance profile (number of drugs) with animal isolates. More resistance was reported in animal than human isolates.

Significance: The results from this study provide an insight into the role of domestic animals as sources of human salmonellosis but cast doubts on the reports that domestic animal *Salmonella* are major donors of resistance genes to human pathogens. The data are relevant to public health and contributes to future risk assessment of AMR in zoonotic *Salmonella*.

P1-01 Potential Internalization of *Escherichia coli* O157:H7 in Lettuce (*Lactuca sativa L.*) by Soil Inoculation

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Introduction: Understanding whether internalization of foodborne pathogens through plant roots occurs or not will be helpful in conducting risk assessments and developing effective interventions to reduce pathogen contamination in produce.

Purpose: (1) to determine whether internalization of *E. coli* O157:H7 through lettuce roots occurs or not; and (2) to determine if differences exist among *E. coli* O157:H7 isolates and lettuce types regarding *E. coli* O157:H7 internalization, survival and growth in and on lettuce plants.

Methods: Iceberg, Romaine and leaf lettuces were grown in sandy soil in an envirotron using two temperature regimes. Soil was inoculated with 5 GFP-labeled *E. coli* O157:H7 isolates individually at 10^6 or 10^3 CFU/g soil when lettuce seedlings were transplanted. Lettuce plants were sampled 2 to 3 times after transplantation and assayed for *E. coli* O157:H7 in soil and in/on shoots and roots.

Results: Results revealed that surface-sterilized leaf and root samples were negative (except for 2 root samples) for *E. coli* O157:H7. Seventeen days after transplantation and inoculation, most leaf surfaces were positive for *E. coli* O157:H7, which was likely due to cross contamination from the inoculated soil. The 26-, 45- and 60-day samplings revealed no *E. coli* O157:H7 on leaf surfaces. Some soil and rhizosphere samples were positive for *E. coli* O157:H7 at 60 days when the trials were terminated.

Significance: Internalization of *E. coli* O157:H7 in lettuce did not occur through the roots; however, the pathogen could survive in soil for at least 60 days. There were no differences among *E. coli* O157:H7 isolates or lettuce types with regard to *E. coli* O157:H7 internalization in lettuce.

P1-02 Comparison of Thermal Tolerance between Outbreak-Associated and Clinical Isolates of *Salmonella* Tennessee in Peanut Butter

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Introduction: A large multistate foodborne outbreak caused by *Salmonella* Tennessee in peanut butter was reported in 2006. Thermal inactivation of three *S.* Tennessee strains associated with the outbreak in peanut butter revealed high heat resistance in these strains.

Purpose: To compare the rates of thermal inactivation in peanut butter of the outbreak strains of *S.* Tennessee with clinical isolates from sporadic cases of *S.* Tennessee.

Methods: Commercial peanut butter was inoculated with *Salmonella* strains and heated to 71, 77, 83, and 90°C. At least three independent trials were conducted at each temperature and for each group of *Salmonella*. The thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (20 min) followed by lower death rates for the remaining cells. The nonlinear Weibull model was used to fit the curves and describe the thermal inactivation of *Salmonella* in the peanut butter.

Results: The calculated minimum times needed to obtain a 5-log reduction at all temperatures for the composited three outbreak-associated strains were significantly higher ($P < 0.05$) compared with those of the 2-strain mixture of clinical isolates of *S.* Tennessee. At 90°C, 41 ± 3 min were needed to reduce the 3-strain mixture of outbreak-associated *S.*Tennessee by 5-log, whereas 26 ± 2 min were needed for the 2-strain mixture of clinical isolates of *S.* Tennessee.

Significance: Results indicated that the outbreak-associated *S.* Tennessee strains were significantly more thermal tolerant than the two clinical isolates of *S.* Tennessee tested. Thermal treatments of peanut butter at 90°C for less than 20 min are not sufficient to kill large populations of *S.* Tennessee in highly contaminated peanut butter.

P1-03 Evaluation of Surface-Sterilization Methods for *Escherichia coli* O157:H7 on Lettuce

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Introduction: Several outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections have been associated with consumption of fresh-cut leafy greens. Questions remain regarding the ability of these pathogens to become internalized within lettuce and spinach. An effective surface-sterilization method for lettuce is needed to study internalization.

Purpose: To identify the best surface-sterilization method for lettuce.

Methods: Iceberg lettuce (*Lactuca sativa L.*) was purchased from a local grocery store and cut into 3 × 3 cm pieces. Lettuce roots were purchased from a local farmer. Leaf pieces and roots were inoculated by immersing in 10^8 CFU five-strain mixture of GFP-labeled *E. coli* O157:H7/ml for 10 min at room temperature. Inoculated samples were put in a laminar flow biosafety cabinet for 30 min before further treatment. Thirteen surface-sterilization methods (including sodium hypochlorite, ethanol, $HgCl_2$, and hydrogen peroxide) were compared for their efficacy in killing/removing *E. coli* O157:H7 on lettuce leaf and root surfaces. Treated samples were washed 5 times with sterile water, and then assayed for *E. coli* O157:H7.

Results: Among the 13 surface-sterilization methods evaluated, *E. coli* O157:H7 was not detected on treated samples by enumeration for 3 treatments, including 20 min with 10,000 ppm sodium hypochlorite

and 2 treatments containing ethanol and $HgCl_2$. There were 2.8 to 4.4 CFU *E. coli* O157:H7/leaf piece or root after surface-sterilization for the other methods. Print and enrichment data were consistent with enumeration results.

Significance: The best surface-sterilization method for lettuce leaves and roots was dipping in 80% ethanol for 10 s, followed by immersion in 0.1% $HgCl_2$ for 10 min.

P1-04 Potential Internalization of *Escherichia coli* O157:H7 in Pre-Harvest Iceberg Lettuce (*Lactuca sativa L.*)

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Introduction: The ability of foodborne pathogens to internalize lettuce, especially under growing conditions, is an important unanswered question in need of elucidation for risk analysis and intervention purposes.

Purpose: (1) to determine the effect of inoculation sites (abaxial vs adaxial leaf surfaces) on survival and internalization of *E. coli* O157:H7 in lettuce; and (2) to evaluate the vulnerability of lettuce at different ages to *E. coli* O157:H7.

Methods: Iceberg lettuce was grown in sandy soil in an envirotron at 23°C during the day and 7°C at night. A 5-strain mixture of GFP-labeled *E. coli* O157:H7 at 10^6 CFU/ml in water and cow manure extract was used as inoculum. Plants were inoculated on the abaxial and adaxial sides of leaf surfaces at 3, 30, 60 days after transplantation and sampled 2–3 times. At each sampling time, *E. coli* O157:H7 in soil and in/on shoots and roots were analyzed.

Results: Twenty-five days after inoculation, 2 of 12 samples were *E. coli* O157:H7 positive on inoculated leaves. No *E. coli* O157:H7 was detected on inoculated leaves at 54 days. All surface-sterilized root and leaf samples were negative for *E. coli* O157:H7 regardless of plant age at inoculation, sampling time, or abaxial versus adaxial side inoculation. Substantially more lettuce leaves inoculated on the abaxial side were *E. coli* O157:H7 positive after 3–25 days than inoculated on the adaxial side.

Significance: Internalization of *E. coli* O157:H7 in iceberg lettuce by leaf inoculation did not occur. Age of lettuce plants did not affect internalization of *E. coli* O157:H7 in lettuce. Inoculated *E. coli* O157:H7 survived longer on the abaxial side of the leaves than on the adaxial side.

P1-05 Pre-Harvest Internalization of Zoonotic Pathogens by Lettuce as Influenced by Environmental Growth Conditions

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Introduction: In recent years, the produce industry has seen an increased number of outbreaks that have been traced to the farm. Potential vehicles for contamination of produce pre-harvest include soil amendments or contaminated irrigation or runoff water. Both surface and internalized contamination have been demonstrated in the laboratory with seeds or seedlings; however, the extent to which internalization occurs in older plants and the environmental growth conditions that may influence contamination internally have not been delineated.

Purpose: To determine: (1) the effect of lettuce plant maturity on pathogen internalization when exposed to contaminated water containing varying populations of pathogens and (2) the influence of plant stress and soil fertility levels on internalization of pathogens by lettuce plants.

Methods: Green leaf lettuce was grown in pots, using either 0:5, 1:5 or 2:5 manure compost:top soil mixtures. Pots were held at 20°C during the day and 7°C at night. An inoculum mixture of GFP-labeled *Escherichia coli* O157:H7 isolates or GFP-labeled *Salmonella* spp. isolates was prepared and added to water to give concentrations of 10^3 or 10^6 CFU/ml. Contaminated water was applied directly to the soil of 3- or 33-day post-transplanted plants (30–50 ml/plant) and a portion of those plants were sampled 3 days later and at 60-days post-transplantation. For a subgroup of plants exposed at 33 days post-transplantation, a reduced watering rate was applied for 2–3 weeks prior to the contamination event. Surface sterilization (ethanol and mercury chloride wash) of samples preceded enumeration of internalized populations.

Results: Neither *E. coli* O157:H7 nor *Salmonella* spp. were internalized into green leafy lettuce. Pathogen internalization was not affected by the level of soil fertility nor did a 2-week period of reduced watering prior to the contamination event induce internalization of pathogens.

Significance: These results suggest that plant internalization via uptake of pathogens from the soil is likely a rare event.

P1-06 Persistence of Enterohemorrhagic and Non-Pathogenic *Escherichia coli* on Spinach Leaves and in Rhizosphere

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Introduction: Outbreaks associated with leafy greens have focused attention on the persistence of *Escherichia coli* O157:H7 on produce. Ecological interactions of *E. coli* O157:H7 and spinach require detailed characterization.

Purpose: Survival of *E. coli* O157:H7 and non-pathogenic *E. coli* was evaluated on spinach plants and in organic soil in a growth chamber.

Methods: Five separate inocula, each containing one strain of *E. coli* O157:H7 and one non-pathogenic *E. coli* isolate (from plants or soil), were grown in water extracts of dairy manure solids. Spinach cultivar 'Whale' was grown in sandy loam soil (3% organic matter). Leaf and soil inocula consisted of: 100 uL in 5 uL droplets on the upper side of leaves and 1 ml in soil used to inoculate individual 4-week old plants with 6.5 log CFU/plant and 7.5 log CFU/200 g soil per plant. Four replicates of each plant shoot and soil sample per inoculum were analyzed on day 0 and weekly for 28 days for *E. coli* O157:H7 and non-pathogenic *E. coli* (by MPN), and for heterotrophic plate counts (HPC).

Results: E. coli O157:H7 was not detected on plant shoots after 7 days but did survive in soil for up to 28 days. Non-pathogenic *E. coli* survived up to 14 days on shoots, and at low levels for up to 28 days. MPN assay was more sensitive than spiral plating in detecting target organisms. HPCs in soil at day 7 were significantly greater than those at day 0, but populations on day 0 and day 28 in soil and on plants were similar ($P > 0.05$).

Significance: Populations of *E. coli* O157:H7 survived for a shorter duration on spinach shoots than in soil. Non-pathogenic *E. coli* were detected intermittently

on spinach up to 28 days. Results suggest survival of these *E. coli* O157:H7 and non-pathogenic strains were similar on leaves and in rhizosphere soil.

P1-07 Attachment of *Escherichia coli* O157:H7 to Intact and Cut Lettuce Surfaces

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Introduction: *Escherichia coli* O157:H7 contamination in produce may occur at any point throughout the food continuum via incidental contact with the organism. To develop effective strategies to minimize the risk of foodborne disease caused by this organism, it is essential to examine initial stages of bacterial attachment to various plant tissues.

Purpose: The purpose of this study was to evaluate the attachment of various *E. coli* O157:H7 strains to types of lettuce surfaces

Methods: Two configurations of coupons: 2-cm disk shaped lettuce coupons (intact surface) and 2 × 0.5-cm strips cut from the mid-vein of leaf at the thickest point near the base of the lettuce (cut surface) were used. Coupons/strips (n = 144) were submerged into four individual *E. coli* O157:H7 strains and stored at 10°C for up to 4 h. Samples were removed periodically and analyzed for loosely attached cells (vortexed for 20 s in PBS/Tween20) and strongly attached cells (homogenized for 20 s using high speed homogenizer).

Results: All strains exhibited preference to attach to cut surfaces rather than intact surfaces. Most strains attached to cut surface at levels 0.5–1.2 log CFU/cm^2 above numbers on intact surface. While all strains were able to attach to both intact and cut surfaces, some strains exhibited significantly higher levels of attachment on cut surfaces. Populations of strongly attached *E. coli* O157:H7 on intact and cut surfaces (5.23 and 5.98 log CFU/cm^2) were significantly different from loosely attached bacterial populations (4.75 and 5.68 log CFU/cm^2), respectively. Most bacterial attachment occurred during the first 15 min of exposure to leaves.

Significance: The study shows the preferential attachment of *E. coli* O157:H7 to cut surfaces. Since *E. coli* O157:H7 binds rapidly to plant tissues, GMP must be in place to avoid cross contamination of other products. Results may aid in understanding the contamination process and in developing more effective disinfection procedures.

P1-08 DSC Impact of Pre-Inoculation Growth Conditions on the Behavior of *Escherichia coli* O157:H7 Inoculated onto Romaine Lettuce Plants and Cut Leaf Surfaces

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Introduction: Inoculum preparation methods have been shown to impact growth or survival of the inoculated organism in a number of food systems, thus complicating direct comparison of results from different studies.

Purpose: To evaluate pre-inoculation growth conditions for impact on *E. coli* O157:H7 inoculated onto leaves of Romaine lettuce plants and cut leaf surface.

Methods: Kanamycin-resistant *E. coli* O157:H7 (human isolate, lettuce outbreak) was grown quiescently or shaken at 37°C to an optical density (OD_{600}) of 0.7 to 1.0 in Tryptic Soy Broth or Agar (TSA), or M9 minimal salts broth or agar. Cells were washed and brought to an OD_{600} of 1.0 in 0.1% peptone and refrigerated for 0 or 18 h. Prepared inoculum was spotted onto cut Romaine lettuce (10 µl; 3 × 10^4 CFU/10 g) or onto leaves of Romaine lettuce plants (20 µl; 3 × 10^6/leaf). Cut lettuce was sealed in 100 cm^2 commercial fresh-cut bags and incubated at 5°C or 20°C. Lettuce plants were held at 23°C for 24 h. Samples were either ground or stomached in 0.1% peptone prior to plating on TSA with 50 mg/l of kanamycin and then incubated at 37°C for 24 h.

Results: For all pre-inoculation growth conditions, populations of *E. coli* O157:H7 increased by 1.5 to 2 log CFU/10 g over 48 h at 20°C and decreased 0.5 to 1.0 log CFU/ 10 g over 5 days at 5°C when inoculated onto cut lettuce. Populations decreased 2.5 to 3.5 log CFU/leaf in 24 h when inoculated onto leaves of lettuce plants. Significant differences ($P < 0.05$) were not observed among any of the pre-inoculation variables tested on either Romaine lettuce plants or cut leaves.

Significance: A range of pre-inoculation growth conditions do not impact the behavior of *E. coli* O157:H7 inoculated onto Romaine lettuce.

P1-09 The Attachment of Shiga Toxigenic *Escherichia coli* to Iceberg Lettuce as Affected by Hydrophobicity and Bacterial Growth Medium

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Introduction: Shiga toxigenic *Escherichia coli* (STEC) are important foodborne pathogens associated with disease outbreaks through contaminated fresh produce such as lettuce and spinach. The attachment of STEC to leaf surfaces may increase their survival, possibly resulting in foodborne disease.

Purpose: The objective of this study was to determine if the hydrophobicity of 9 STEC strains (including serotypes O157 (5 strains), O26, O91, O111, O165) and 1 non-STEC *E. coli* strain influences their ability to attach to intact and bruised lettuce leaves following growth in minimal and nutrient media.

Methods: Cultures grown in Tryptone Soya Broth (TSB) or Minimal Broth Davis with glucose (MBDG) at 37°C for 19 ± 1 h were washed and suspended in sterile deionized water (SDW) to approximately 10^8 CFU/ml. Intact and experimentally bruised lettuce pieces were immersed in 10 ml of the bacterial suspension for 30 min at room temperature (20–24°C) and the percentage of attached cells was calculated, using viable counts on Tryptone Soya Agar (TSA). The hydrophobicity of STEC was measured with Hydrophobic Interaction Chromatography (HIC) with phenyl-sepharose as the stationary phase.

Results: Attachment of all STEC grown in both nutrient and minimal media was significantly higher on bruised lettuce tissue ($P < 0.05$) than on intact tissue. No correlation was found between the hydrophobicity of STEC isolates and attachment to intact or bruised lettuce leaves ($R^2 < 0.7$). The hydrophobicity of four of the serotype O157 was significantly higher when they were grown in MBDG rather than TSB, while the non-STEC strain was significantly more hydrophobic when grown in TSB. No other differences between strains and growth media were apparent.

Significance: Hydrophobicty of STEC may not play a role in their persistence on lettuce, but bruising of lettuce is likely to increase the likelihood of disease transmittance by these pathogens.

P1-10 Use of Fluorescent Dyes for Visualization of Bacterial Attachment to Lettuce Leaves

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Introduction: Produce safety research often involves the use of gfp-labeled bacterial cells for ease of visualizing the attachment of the targeted cells to produce tissues. This genetic manipulation, frequently tied to plasmids coding for antibiotic resistance, results in changes in cell physiology which could have unintended effects on bacterial interaction with produce tissues.

Purpose: The purpose of this study was to develop a non-genetically labeled fluorescent microorganism.

Methods: An overnight culture of *Escherichia coli* O157:H7 RM6044 (Tryptic Soy Broth, 37°C) was centrifuged and washed in sterile deionized water (dH_2O) once, then resuspended in dH_2O. SYTO 9 or 85 dye was added to the culture, which was incubated at 4°C for 3 h. The culture was centrifuged at 9000 rpm for 2 min, washed twice with dH_2O, resuspended in dH_2O, and incubated at room temperature (RT; with or without light) and 4°C (without light) for up to 7 days. Also, the cut bases of Romaine lettuce leaves were immersed in SYTO 9-stained *E. coli* O157:H7 (8 log CFU/ml) for up to 5 days at 4°C. All samples were observed via fluorescence, confocal and scanning electron microscopy.

Results: All stained cultures remained viable and fluorescent following 15 days of storage at RT and 4°C. Unlike cells stained with SYTO 9, SYTO 85-stained cells exhibited rapid photo bleaching during imaging. Colonization of romaine lettuce leaves by the stained *E. coli* O157:H7 cells was easily visualized via fluorescence microscopy. Cells forming a biofilm on the leaf were further examined via confocal and SEM imaging. No internalized *E. coli* O157:H7 were observed in romaine lettuce leaves.

Significance: This easy method of staining, which does not require specialized equipment or training, allows for the observation of static bacterial population attachment to produce surfaces.

P1-11 Efficacy of Chlorine Concentration and Acidic Electrolyzed Water in Decontaminating Lettuce Leaves Artificially Inoculated with *Escherichia coli* O157:H7

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Introduction: Consumption of lettuce has been linked to outbreaks of foodborne illnesses and recalls due to contamination with *Escherichia coli* O157:H7. Reduction of this risk requires the development of an effective and easily implemented decontamination process.

Purpose: The purpose of this study was to compare the efficacy of industrial chlorine wash (20 ppm) to that of 200 ppm chlorine and electrolyzed water (50 ppm) treatments in reducing populations of *E. coli* O157:H7 on artificially inoculated lettuce.

Methods: Fresh-cut leaves of Romaine and Iceberg lettuce were inoculated by immersion in water containing *E. coli* O157:H7 (8 log CFU/ml) for 5 min and dried in a salad spinner. Leaves were then washed for 2 min, immediately or following 24 h of storage at 4°C, using: (1) deionized water; (2) acidic electrolyzed water (50 ppm chlorine and pH 2.6); (3) 20 ppm chlorine; (4) 200 ppm chlorine. Following 2 min treatment, samples were blended in neutralizing buffer (1:3), serially diluted, and surface plated on the selective medium CT-SMAC.

Results: There was no difference in the levels of *E. coli* O157:H7 on Romaine or Iceberg leaves at 0 and 24 h. The washing treatment using 200 ppm chlorine resulted in the greatest reduction in *E. coli* O157:H7 populations (1.84 ± 0.53 and 1.07 ± 0.50 log CFU/g at 0 and 24 h, respectively) as compared to the other wash treatments used (<1 log CFU/g reduction).

Significance: The industrial chlorine (20 ppm) wash was shown to be ineffective in inactivating *E. coli* O157:H7 cells on lettuce. While the 200 ppm chlorine wash treatment was capable of achieving a nearly 2 log reduction, the process needs to be optimized to meet the FDA 5 log requirement.

P1-12 Influence of Biofilm-Forming Bacteria on Association of Hepatitis A Virus with Lettuce

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Introduction: Viral foodborne illness outbreaks have been associated with produce, including leafy greens. The potential accumulation of viruses in biofilms in drinking water systems has been reported; however, the influence of bacteria on the association and viability of viruses on plant tissue has not been thoroughly evaluated.

Purpose: This study was undertaken to quantify the transfer and survival of Hepatitis A virus (HAV) on lettuce leaves, alone and in the presence of biofilm-forming bacteria.

Methods: Ultraviolet light-treated iceberg lettuce was inoculated with HAV (approximately 6 log $TCID_{50}$/ml) alone or in a bacterial cocktail (1×10^7 CFU/ml) of *Pseudomonas fluorescens* 13525 and *Escherichia coli* O157:H7 (5 strains) in sterile deionized water. After 50 h at 15°C, the lettuce was washed twice, and the wash water and lettuce were analyzed for HAV. Viral RNA was quantified by reverse-transcriptase, real-time PCR with a standard curve for pure HAV. HAV viability was determined in cell culture with fetal rhesus monkey kidney cells. Cytopathic effect was calculated by the Reed and Muench method.

Results: As determined by PCR, 2.4×10^7 and 3.4×10^6 virus particles were enumerated from wash water from lettuce inoculated with HAV alone or HAV with bacteria, respectively. Fewer HAV particles were detected from lettuce tissue with 3.0×10^5 and 6.7×10^5 particles from HAV alone and HAV with bacteria, respectively. Viable HAV were detected in wash water (approximately 4.2 log $TCID_{50}$/ml) and lettuce (approximately 2.4 log $TCID_{50}$/ml) for both samples inoculated with HAV alone or with bacteria.

Significance: The simultaneous contamination of HAV and biofilm-forming bacteria on lettuce did not preclude or enhance virus association with the lettuce in early biofilm formation. The fate of virus introduced after the establishment of biofilm and prolonged storage is worthy of further investigation.

Poster

P1-13 Development of a Simple Model System to Internalize *Escherichia coli* O157:H7 in Lettuce Leaves and to Evaluate Release into Wash Waters from Cut Surfaces

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Introduction: During 1995–2006, there were 22 outbreaks of foodborne disease caused by *Escherichia coli* O157:H7 associated with consumption of fresh produce items. In some outbreaks, it has been suggested, bacteria may be internalized and not surface-associated.

Purpose: The aim was to develop a simple in vitro model to internalize *E. coli* O157:H7 in lettuce leaves. Such a model system could be used as a research tool to investigate the effect of intervention strategies (e.g., wash water sanitation) or, as described below, to assess methods to detect internalized bacteria leached into residual wash waters from cut surfaces.

Methods: Internalization: Inner leaves from heads of fresh Romaine lettuce were cut to size, and cut ends were placed in covered suspensions of 10^9 CFU/ml *E. coli* O157:H7 F4546 in 40 ml BPB. Individual leaves were held for 1, 2 and 3 days at room temperature to assess uptake. Leaf surfaces were washed with 1% $AgNO_3$ solution to inactivate potential surface contaminants, and homogenized; bacteria were enumerated on SMAC agar. Scanning electron microscopy (SEM) was used to confirm internalization at cut surfaces.

Detection: Pieces were cut from the leaves in cross-section at 2 cm intervals, and various numbers of pieces (1–4) from different parts of the leaf were spun for 1 min in 900 ml DI water in salad spinners to simulate the release of water from lettuce in a centrifugal de-watering step. The collected water was added to 100 ml 10× EHEC Enrichment Broth (EEB) and enriched overnight at 37°C. Cultures were confirmed by streaking to SMAC agar.

Results: Approximately 10^2–10^3 CFU/g *E. coli* O157:H7 were internalized in lettuce leaves. There was no statistical significant difference between the numbers internalized after 1, 2 or 3 days. SEM gave evidence of successful internalization. Positive results were observed after enrichment of rinse waters, showing that internalized *E. coli* O157:H7 at exposed surfaces leached into the waste water stream. The more pieces spun, the greater the likelihood of detection.

Significance: A simple model has been developed to internalize *E. coli* O157:H7 in lettuce leaves. Data suggest that bacteria may be released into the waste water stream in a simulated de-watering step which may serve as a useful indicator of potential batch contamination.

P1-14 Survival and Growth of *Escherichia albertii* on Fresh-Cut Lettuce Stored at Various Temperatures

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Introduction: Escherichia albertii is a potential foodborne pathogen because of its ability to cause diarrheal disease. The behavior of the pathogen in foods, especially produce commodities, has not been widely examined.

Purpose: The behavior of three strains of *E. albertii* on fresh-cut iceberg lettuce stored at different temperatures was evaluated.

Methods: Three nalidixic acid (Nal)-resistant strains of *E. albertii* (9194, 10457, and 19982) were spot inoculated on pieces of lettuce (9 cm^2) with 3.22, 2.63, and 5.28 log CFU/cm^2, respectively, and allowed to dry for 1 h before being sealed in a petri plate with a wet piece of filter paper and stored at 5 and 15°C (up to 7 days), or 30°C (up to 2 days). Samples were homogenized and then enumerated on selective RMACN (rhamnose MacConkey Agar supplemented with Nal) at appropriate times for enumeration. Two replicate experiments of each strain on cut lettuce ($n = 4$) were performed, and significant differences ($P < 0.05$) between strains were determined.

Results: After 7 days at 5°C, strains 10457 and 19982 decreased by 0.78 and 1.56 log CFU/cm^2 of lettuce, respectively, while strain 9194 increased by 0.52 log CFU/cm^2. After 7 days at 15°C, numbers of strain 9194 were significantly greater (5.16 log CFU/cm^2) than those of strains 10457 and 19982 (4.48 and 4.28 log CFU/cm^2), respectively. At 30°C, numbers of strain 9194 (6.24 log CFU/cm^2) were significantly greater than those of strain 10457 (5.82 log CFU/cm^2) after 2 days. After 7 days of storage, all strains grew by at least 0.70 log CFU/cm^2 and 2.31 log CFU/cm^2 at 15 and 30°C, respectively.

Significance: Strain 9194 showed marginal growth when stored at 5°C, indicating that this strain of *E. albertii* may be more persistent at low temperatures than other strains. As expected, all strains of *E. albertii* grew on lettuce at abusive temperatures. This is the first examination of *E. albertii* on fresh-cut produce.

P1-15 Visualization of Attachment and Internalization of a Bioluminescent Derivative of *Escherichia coli* O157:H7 ATCC 43895 on Lettuce Leaves

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Introduction: Escherichia coli O157:H7 has been the cause of an increasing number of foodborne outbreaks involving fresh lettuce and spinach. The potential for fresh produce contamination by *Escherichia coli* O157:H7 is of concern because these vegetables are consumed raw.

Purpose: The purpose of this research was to determine if *E. coli* O157:H7 (curli) ATCC 43895 expressing a constitutive curli phenotype could attach to and be internalized by lettuce.

Methods: Lettuce leaves were attached to the rear plate of a flow cell using cyanoacrylate in both dorsal and ventral configurations and incubated with 10^5 CFU/ml of a bioluminescent derivative of *E. coli* O157:H7 (curli) for 60 min. Following incubation, the flow cell was drained and the lettuce was rinsed twice with fresh media before being incubated for 10 min at 37°C to ensure adequate light production. The samples were then visualized under a photon counting camera for 10 min to determine the spatial patterns of attachment.

Results: E. coli O157:H7 (curli) lux attached to and followed the veins of the lettuce leaves, particularly along the portions connected to the main vein of the lettuce. *E. coli* O157:H7 (curli) lux also traveled to the interior of the leaves and continued to bioluminesce after the lettuce was sprayed with 70% ethanol.

Significance: Based on the results collected in this study, *E. coli* O157:H7 (curli) lux attached to the veins of the lettuce and remained active after lettuce decontamination. This work may help to explain some of the occurrence of foodborne illness associated with leafy greens and suggest ways to improve food safety during processing.

P1-16 Efficacy of Antimicrobial Agents to Reduce Transfer of *Escherichia coli* O157:H7 on Lettuce Pieces

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Introduction: Antimicrobial agents are often added to water used in the flume conveying or washing of fresh fruits and vegetables. The addition of these agents reduces the number of microorganisms in fruit and vegetable process water. Reducing the number of microorganisms in recycled process water helps prevent it from becoming a vector of cross contamination. Antimicrobial chemicals in process water will also cause a reduction of microorganisms on the surface of fruits and vegetables.

Purpose: The objective of this study is to evaluate the effectiveness of antimicrobial agents in controlling the transfer of *Escherichia coli* O157:H7 ATCC 43895 inoculated produce in simulated process water under organic challenged conditions.

Methods: A general design of experiment was conducted with 2 factors with multiple levels (2 chlorine products and 2 peracids for antimicrobials; and 10, 20 and 30 ppm concentrations of active) with water as the control. A piece of lettuce leaf was spot inoculated with 100 µL of rifampicin-resistant *E. coli* O157:H7 ATCC 43895 and allowed 2 h for attachment. Antimicrobial solution for each concentration was prepared with lettuce slurry to simulate an organic challenge. Five uninoculated lettuce pieces were placed in the beaker, followed by an inoculated piece. The entire system was agitated for 1.5 min. Inoculated and uninoculated lettuce pieces were removed individually and neutralized. Survivors were enumerated on brain heart infusion agar with rifampicin. The antimicrobial solution was also plated onto the same agar for survivors.

Results: The chlorine had more *E. coli* O157:H7 survivors in the solution and on lettuce compared to the peracids ($P < 0.05$). For the peracids at 20 and 30 ppm, <1 $\log_{10}$ survivors were observed from antimicrobial solution and uninoculated lettuce pieces. Approximately 3.5, 3.5, and < 1 $\log_{10}$ CFU/ml was recovered from water, 30 ppm chlorine, and 30 ppm peracid fluids, respectively, when a lettuce leaf inoculated with 5 $\log_{10}$ CFU/ml of the organism was mixed with 5 uninoculated leaves and 10% lettuce juice. Furthermore, 2.5, 2.6, and <1 $\log_{10}$ CFU/ml were recovered from uninoculated leaves following the same treatment parameters respectively. Both the chlorine formulations gave results similar to results for the water control.

Significance: Peracid, but not chlorine, was effective in controlling the transfer of *E. coli* O157:H7 from inoculated pieces to uninoculated pieces in an organic challenged system.

P1-17 Survival of *Salmonella* and *Escherichia coli* O157:H7 in Peanut Butter under Different Storage Temperatures

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Introduction: In 2007, a total of 628 persons infected with an outbreak strain of *Salmonella* serotype Tennessee were reported from 47 states in United States. The outbreak was associated with consumption of contaminated peanut butter.

Purpose: This study was undertaken to determine the survival of *Salmonella* and *Escherichia coli* O157:H7 in peanut butter.

Methods: Peanut butter was inoculated with either antibiotic resistant *Salmonella* or *Escherichia coli* O157:H7 at a concentration of 4.78 log CFU/g and 5.56 log CFU/g, respectively. Peanut butter samples were analyzed on the first and third days post inoculation, then monitored continuously after every week for a total of 15 weeks.

Results: Populations of *Salmonella* in inoculated peanut butter samples stored for 15 weeks at 25°C and 9 weeks at 4°C decreased to 2.17 log CFU/g and 3.76 log CFU/g, respectively. The *Escherichia coli* O157:H7 contaminated peanut butter stored for 15 weeks at 25°C and 9 weeks at 4°C decreased to 2.64 log CFU/g and 1.18 log CFU/g, respectively. Populations of *Escherichia coli* O157:H7 decreased significantly more ($P < 0.05$) than *Salmonella* during the storage period.

Significance: These results demonstrate that post-process contamination of peanut butter with *Salmonella* and *Escherichia coli* O157:H7 may result in survival of these pathogens during the shelf life at 25°C and 4°C. Foodborne pathogens can be introduced into the production environment and this clearly raises concern about the safety of peanut butter and other spread products.

P1-18 Environmental Investigation of a Restaurant's *Escherichia coli* O157:H7 Outbreak Linked to Iceberg Lettuce

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Introduction: In December of 2006, the California Department of Public Health was notified of a multi-state *Escherichia coli* O157:H7 outbreak linked to iceberg lettuce. Approximately 80 individuals were sickened — no deaths were reported. The US Food and Drug Administration, working with Minnesota and California public health officials, traced the lettuce to growing areas in California — Santa Maria (Central Coast) and Buttonwillow (Central Valley).

Purpose: Based on epidemiological and traceback data, the California Food Emergency Response Team (CalFERT) was mobilized. An environmental investigation was launched focusing on outbreak-control measures, traceback to farms, and identification of possible sites of contamination at the farm level.

Methods: CalFERT examined 11 growing fields, interviewed growers, examined harvesters, and coolers and collected 251 environmental samples, including

water, soil, swabs, feces, and product. Samples were tested for *E. coli* O157:H7 and its shiga toxins. Positive isolates were further analyzed to determine if they genetically matched the outbreak strain. For one implicated farm in Buttonwillow, further investigation was warranted due to (1) identification of the farm as one that supplied product to the implicated processor during the time period in question, (2) the farm's close proximity to two dairies and (3) an environmental sample at a nearby dairy matching the outbreak strain. Irrigation and dairy effluent conveyance systems appeared to be combined into a complex piping network on this farm, raising concerns about microbial cross contamination potential between fields of lettuce and nearby dairies.

Results: Of 251 samples collected, 32 (~13%; all from the farm in Buttonwillow) were positive for *E. coli* O157:H7. Ten of the 32 positives (~31%) genetically matched the outbreak strain; six (60%; one swab, one water, three water and sediment, and one soil) were collected close to an implicated lettuce growing field, and four (40%; one swab and three water) came from two dairies near suspect growing fields.

P1-19 Survival of Attenuated *Escherichia coli* O157:H7 ATCC 700728 in Field-Innoculated Lettuce

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Introduction: Behavior of *E. coli* O157:H7 in lettuce has been mainly studied on cut leafy greens. While these data are useful in establishing the survival of *E. coli* under post-processing conditions, they provide little information on the association of *E. coli* O157:H7 on growing lettuce plants.

Purpose: To evaluate the impact of inoculum concentration and irrigation method on the survival of *E. coli* O157:H7 in field-inoculated lettuce.

Methods: A split plot design with three replicates was used to evaluate two main treatments: drip and overhead irrigation. Rifampicin-resistant attenuated *E. coli* O157:H7 (ATCC 700728), harvested from Tryptic Soy Agar (TSA) plates and suspended in 0.1% peptone, was inoculated by spraying onto 4-week old lettuce plants at target inoculum concentrations of 5 and 7 log CFU/plant. *E. coli* O157:H7 was recovered by stomaching, standard enumeration and filtration on TSA with 50 µg/ml of rifampicin. When counts were below the limit of detection (1 cell/plant) prevalence was determined by enrichment of entire plants in TSB with 50 µg/ml rifampicin.

Results: Recovered levels of *E. coli* O157:H7 700728 were 3 and 4 log CFU/plant immediately after inoculation and 1 and 3 log CFU/plant 1 h after inoculation. After 2 days, 2 and 8 of 30 plants yielded counts for the low and high inoculum levels, respectively. Countable populations ranged from 1 to 179 cells per plant. At day 7, 14, and 21 plants were scored positive or negative based on enrichment of individual heads of lettuce. Positive samples were detected at day 14 (1 and 7 of 30 for low and high inoculum levels, respectively), but none of 90 samples were positive at day 21. Differences were not detected between irrigation methods.

Significance: A better understanding of factors that influence *E. coli* O157:H7 survival in lettuce fields will help design strategies to reduce pre-harvest contamination.

P1-20 DSC Evaluation of Chemical Disinfection Treatments to Inactivate *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Mexican Spinach

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Introduction: The recent *E. coli* O157:H7 outbreak associated with the consumption of fresh spinach resulted in three deaths, numerous hospitalizations, and economic losses to the fresh greens industry in the United States. *Salmonella* and *L. monocytogenes* have also been linked to outbreaks due to the consumption of raw produce, including food items imported from Mexico and Latin America. Chemical disinfection is widely used as an intervention step to control pathogenic microorganisms.

Purpose: Our objective was to evaluate the effect of chlorine, peroxyacetic acid, and colloidal silver against *Listeria monocytogenes* and *E. coli* O157:H7 on fresh Mexican spinach.

Methods: Rifampicin-resistant strains of *E. coli* O157:H7 (four) and *L. monocytogenes* (five) were pooled separately for each pathogen to a concentration of approximately 10^9 and 10^8 CFU/ml, respectively. Spinach pieces (5 × 5 cm) were spot-inoculated with 100 µl and then subjected to chemical treatments ith chlorine (200 mg/l), peroxyacetic acid (80 mg/l), and colloidal silver (1.4 mg/l) for 5 min. Finally, the spinach was neutralized and survivors were recovered on Tryptic Soy Agar supplemented with rifampicin (100 mg/l).

Results: Reductions for *L. monocytogenes* with peroxyacetic acid, chlorine and colloidal silver were 3.8, 3.0, and 1.4 log CFU/spinach sample, respectively. The same trend was observed for *E. coli* O157:H7: 3.7, 1.9 and 1.0 log CFU/spinach sample, respectively. There was no significant difference reduction ($P > 0.05$) of *L. monocytogenes* between peroxyacetic acid and chlorine. In general peroxyacetic acid was the most effective treatment for inactivation of both pathogens on spinach.

Significance: These results showed that peroxyacetic acid could be an alternative treatment for the elimination of foodborne pathogens on produce in Mexico.

P1-21 Effects of Temperature Abuse and Subsequent Cold Storage on Natural Microflora and *Escherichia coli* O157:H7 on Spinach

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Introduction: Previous studies have shown that *E. coli* O157:H7 inoculated onto fresh produce at relatively high doses (10^2–10^8 CFU/g) does not grow at temperatures below 8°C. Few reports have focused on how storage at moderately elevated temperatures impact the growth of low levels of the bacteria (< 1 CFU/g) on fresh produce and the fate of those bacteria after shifting the produce back to regular storage temperature.

Purpose: This study examined the effects of short periods of elevated 'abusive temperature' treatments and subsequent cold storage (4°C) on the growth and survival of low levels (< 0.5 CFU/g) of *E. coli* O157:H7 and indigenous flora on fresh spinach.

Methods: *E. coli* O157:H7 (< 0.5 CFU/g) was inoculated on fresh spinach. An MPN-based PCR assay (MPNPCR) was used to follow the fate of the bacteria after brief (6–36 h) treatments at various temperatures (10°C, 15°C, 23°C, and 30°C), and how the bacterial numbers were affected after the samples were moved back to normal storage temperature (4°C). Growth of indigenous mesophilic heterotrophic bacteria was also monitored, using a microplate-based MPN procedure.

Results: Significant effects of elevated temperature ($P < 0.05$) were observed in *E. coli* O157:H7 populations after only 6 h at 23° and 30°C, 12 h at 15°C, and 36 h at 10°C. While these effects were not substantial for bacterial populations treated at 10°C and 15°C, *E. coli* O157:H7 grew with doubling times of 3.6 h at 23°C and 2.3 h at 30°C during the treatment time. When the inoculated spinach samples were returned to normal storage temperature (4°C), *E. coli* O157:H7 persisted for up to 2 weeks. Indigenous mesophilic heterotrophic bacteria had different growth profiles during treatment and storage.

Significance: Given the low dose needed for *E. coli* O157:H7 infection, these results have significant implications for how producers and consumers should approach the storage and preparation of fresh produce.

P1-22 DSC Characterization of Seasonal Diversity of the Spinach (*Spinacea oleracea*) Phyllosphere Microbial Community, Using Culture and Non-Culture Dependent Techniques

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Introduction: Several recent foodborne pathogen outbreaks are associated with leafy greens. By identifying the native microbial communities on surfaces of leafy greens, we may begin to elucidate the mechanisms by which bacteria, including foodborne pathogens, survive on edible leaves. It is likely that the native microflora could have a role in the establishment and survival of pathogens.

Purpose: The purpose of this work was to determine changes in microbial diversity of spinach leaves as related to the time of harvesting and the spinach cultivar.

Methods: Three spinach cultivars (Monza, Menorca, Unipack) were organically grown during Fall, 2007 at the Virginia Tech Experimental Research Farm. Spinach leaves (≤ 10 cm) were harvested during the first week of October, November and December. Aerobic plate counts were performed with minimal media and potato dextrose agar for a period of 16 days after harvest. Total microbial DNA was isolated directly from spinach leaves for each cultivar and harvest and 16S rRNA genes were amplified, using universal primers. Denaturant gradient gel electrophoresis (DGGE) was performed on the fragments and dendrograms constructed to study the differences among the three harvest periods and cultivars.

Results: Total counts and colony morphology of culturable epiphytic microbial population showed significant differences ($P > 0.05$) among the three cultivars and the three different harvest periods. Microbial counts were influenced by the leaf surface, with higher microbial counts being found on the Unipack (savoy) leaves than on the Menorca (flat) leaves. DGGE patterns showed that samples with higher microbial counts also correspond to samples with higher diversity. However, no significant differences in DGGE patterns were seen amongst the cultivars.

Significance: Cultivar and time of harvest influence the microbial community.

P1-23 Epiphytic Bacteria and Survival of *Escherichia coli* O157:H7 on Spinach

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Introduction: Minimally processed vegetables have been associated with outbreaks of disease caused by *Escherichia coli* O157:H7. Farm management practices might influence the counts and composition of epiphytic populations, including lactic acid bacteria (LAB), and thus impact the survival of pathogens on leafy vegetables.

Purpose: This study determined the total counts of LAB and generic aerobic bacteria (CFU/g) on spinach from various sources. In addition, the survival of an inoculated green fluorescent *Escherichia coli* O157:H7 strain was investigated.

Methods: Serial dilutions of fresh homogenized spinach samples were spread-plated on non-selective Luria-Bertani agar for determination of total aerobic bacteria and *E. coli* O157 and on Mann Rogosa and Sharpe's agar for LAB.

Results: Counts of generic aerobic bacteria were different among lots of product ($P = 0.002$). However, total LAB counts were similar ($P = 0.15$). Total bacteria counts were negatively correlated with *E. coli* O157:H7 survival (rho = -0.6; $P = 0.01$).

Significance: Variations in epiphytic microbial populations present on spinach grown under different conditions (geography, seasonality, farm management) may contribute to the differential survival rate of *E. coli* O157:H7 on this product.

P1-24 DSC Reduction of *Escherichia coli* O157:H7 in Fresh Commercial Spinach by Lactic Acid Bacteria

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Introduction: Outbreaks of foodborne illness in produce have led to the development of intervention methods. Lactic Acid Bacteria (LAB) as a treatment method may provide a means of reducing pathogenic contaminants in fresh produce.

Purpose: This study was conducted to determine the effectiveness of LAB as an intervention to reduce *E. coli* O157:H7 on fresh spinach leaves.

Methods: Fresh spinach leaves were inoculated with 7.0 log CFU/g of *E. coli* O157:H7. Half of the samples were inoculated with an LAB cocktail consisting of NP51, NP35, NP7 and NP 3 to yield a population of 1×10^7 CFU/g on the leaves of spinach and the other half with an equivalent volume of BPW. The samples were incubated at 4°C and 25°C and sampled at 0, 2, 6, 12, and 24 h. Samples were plated onto MacConkey's agar to determine *E. coli* populations.

Results: The amount of *E. coli* O157 in treated samples containing the LAB did not increase significantly over time at either temperature. At 4°C, the control samples increased to 7.9 and 8.0 log CFU/g after 12 and 24 h, respectively while the treated samples were significantly lower at 6.0 and 5.0 log CFU/g. Initial

populations of *E. coli* O157 on control samples held at 25°C increased to 7.82 and 8 $\log_{10}$ CFU/g after 12 and 24 h, respectively, while no *E. coli* O157 was detected on the LAB treated samples after 12 and 24 h at this temperature.

Significance: These data indicate that treatment of spinach with LAB could improve the safety of this product by controlling *E. coli* O157:H7.

P1-25 The Effect of Different Refrigerator Storage Sites on the Proliferation of Spoilage and Indicator Bacteria on Bagged Spinach

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Introduction: Proper storage and cleaning by consumers are essential in the prevention of foodborne disease, as many cases of foodborne disease are caused by home contamination. Bagged spinach is stored in home refrigerators for several days, with small portions being removed as needed.

Purpose: The study determined if temperature differences within a household refrigerator would affect the proliferation of spoilage and safety indicator bacteria growing on bagged spinach.

Methods: Bagged spinach was stored at three different locations in a refrigerator (top shelf, door, and bottom crisper). At each site, iButtons were placed and programmed to take temperature readings at every half hour for 12 days. Every three days, spinach samples were removed and stomached. Dilutions were plated on Luria Agar, Tergitol 7 agar, and MRS Agar. The plates were incubated for 48 h at 30°C.

Results: The average daily temperature was different at each location in the refrigerator, with the middle shelf of the door having the lowest daily temperature, 5.7°C, and the top shelf having the highest temperature, 8.2°C ($P = 0.0009$). While these temperatures differed, the aerobic plate, lactic acid bacteria, and coliform counts did not differ between the three locations after 12 days ($P > 0.05$). At each location, aerobic plate count increased over 12 days ($P < 0.05$). The lactic acid bacteria count also increased over the time of the study for spinach present on the top shelf of the refrigerator ($P = 0.025$).

Significance: This study showed that there are differences in temperature at different locations within household refrigerators, but these temperatures did not impact the growth of microorganisms growing on bagged spinach. However, the temperature at each location did not prevent the proliferation of bacteria on the spinach plants but was low enough to prevent significant increase in coliform growth.

P1-26 DSC Effect of Lactic Acid Producing Bacteria on the Sensory Characteristics of Fresh Spinach

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Introduction: In recent years, fresh spinach has been implicated as a vehicle for *Escherichia coli* O157:H7 transmission. Numerous studies have identified the ability of lactic acid bacteria (LAB) to reduce the presence of *Escherichia coli* O157:H7 in food products, and the application of LAB cultures as a post-harvest intervention should be considered.

Purpose: To determine if the metabolic byproducts produced by LAB could potentially affect the sensory characteristics of fresh spinach and lead to consumer rejection.

Methods: A triangle test was utilized to determine if a statistically significant difference exists when LAB cultures are applied to fresh spinach. Two spinach samples were rinsed with tap water and considered to be identical. The remaining spinach sample was rinsed with a four-strain LAB culture at a concentration of 10^8 CFU/g. Forty panelists were chosen at random and presented with the three spinach samples simultaneously in a triangle test. The order in which the samples were presented to panelists was randomized in order to reduce bias. Each participant was asked to evaluate each sample from left to right and identify the one sample they perceived to be different.

Results: A total of 16 out of the 40 panelists identified the LAB spinach sample as being different, which is not considered significant.

Significance: These results indicate that a statistically significant difference does not exist with the application of LAB cultures in fresh spinach ($\alpha = 0.05$, 0.01) and that the use of LAB as a post-harvest intervention may be suitable from a consumer acceptance standpoint.

P1-27 Environmental Contamination of Spinach Placed in Close Proximity to Cattle Feedyard Operations

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Introduction: Spinach is exposed to multiple environmental factors throughout the growing process, and the presence of cattle feedyards within the vicinity of spinach production may have an impact on spinach contamination.

Purpose: To determine if the environment impacts the microbial contamination of spinach in close proximity to cattle feedyards.

Methods: Whole spinach bundles were obtained from a local grocery store and tested initially and after 6 and 24 h of exposure for total coliforms, generic *E. coli*, *E. coli* O157:H7 and *Salmonella*. The twelve bundles were secured onto tables in the upright position at two different locations at a local feedyard. Six were placed in the southwest corner under the loading chute, while the remaining six bundles were located on the far east side of the feedlot approximately 100 yards from the loadout area. Both locations were chosen because of their exposure to a great deal of dust generated by the feedlot cattle.

Results: All samples tested negative for the presence of *E. coli* O157:H7 and *Salmonella*, coliform and generic *E. coli* numbers were increased by environmental exposure. Total coliforms on samples from the east side of the feedlot did not change after six h, but increased significantly by 0.6 logs after 24 h, from 0.9 logs to 1.5 logs. Similarly, the samples located on the southwest side of the feedlot increased by 1.6 logs and 3.0 logs after 6 and 24 h, respectively. Generic *E. coli* did not increase after 6 h in samples located on the east side of the feedlot, but increased 0.9 log cycles after 24 h, from 0 logs to 0.9 logs.

Significance: Results indicate that the environment has the potential to contaminate spinach located in close proximity to cattle areas and that control of the environmental conditions could prevent contamination of vegetable crops.

Poster

P1-28 Evaluation of Growth Kinetics of *Escherichia coli* O157:H7 on Bagged Spinach in Relation to Consumption Decisions Based on Visual Quality and Off-Odors

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Introduction: Low levels of contamination of *Escherichia coli* O157:H7 on leafy greens is held to be sufficient to cause illness. Multiplication under sub-optimal temperatures could occur prior to a clear aversion to consumption by sensible and attentive consumers.

Purpose: To evaluate the comparative growth of commensal *E. coli* and *E. coli* O157:H7 on bagged spinach in simulated distribution in relation to presumptive consumer-based consumption decisions.

Methods: A composite inoculum of *Escherichia coli* O157:H7 kan:gfp and commensal *Escherichia coli* was prepared in BPW to log 2.6 CFU/ml. Fresh spinach was immersed 1 min, excess liquid removed, and leaves held at RT for 1 h. Replicates of 45 g of leaves were placed in commercial film. Sealed bags were placed at 20, 15, 10 and 5°C for 0, 5, 10 or 14 days. Spinach was evaluated for indigenous coliforms, inoculated strains, visual quality, hue and chroma, head-space gases, off-odors, ammonia, differential populations on sound versus decayed tissue, and consumer acceptability.

Results: Growth/survival of *E. coli* was proportional to storage temperature. At 5°C, cells remained recoverable for at least 10 days. At 5°C, after 14 days, *E. coli* O157:H7 was undetectable by plating but detected by enrichment (25 g in 225 ml mEHEC + qRTPCR). At 15°C, *E. coli* O157:H7 increased 2.6 log CFU/g in 24 h. Commensal *E. coli* were typically 10 fold greater in population than *E. coli* O157:H7. There were no growth differences between poor and good quality leaves. No other parameter was correlated with population. More than 80% of the consumers (intercept survey; n = 52) answered they would consume spinach after removing poor quality leaves.

Significance: Results clearly demonstrate rapid growth potential of *E. coli* on packaged spinach under sub-optimal temperatures. Visual quality was not a useful indicator of risk. Educational campaigns are needed all the way to the point of consumption.

P1-29 Characterization of Microbial Content of Organic and Conventional Produce in Maryland Relative to Production Practices and Inputs

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Introduction: Production inputs/practices such as soil amendments, water quality, animal intrusion, and human activity can influence microbial quality of fresh produce. Limited data on production practices relative to microbial/pathogen content of fresh produce are currently available for either organic or conventional farms in the Mid-Atlantic region, some of which market directly to consumers.

Purpose: Microbial content of seasonal produce grown during 2006–2007 relative to various production practices/inputs was evaluated, with emphasis on *E. coli* (generic and pathogenic), *Salmonella* spp., and *Listeria* spp.

Methods: Produce (n = 335), water (n = 66), and soil (n = 335) samples from tomatoes, leafy greens, strawberries, green onions, and herbs were collected from matched pairs of organic and conventional farms and analyzed for *E. coli*, *Salmonella*, and *Listeria* spp. *E. coli* were confirmed by biochemical and FAME profiles and further screened by multiplex PCR for specific detection of *stx*1, *stx*2, and *eae* genes of *E. coli* O157:H7. Farm practices at each site were obtained by interviews, on-site observations, and written responses from participants.

Results: Confirmed isolates of *E. coli* included 34 and 46 soil and 5 and 15 commodity samples in 2006 and 2007, respectively. No EHEC strains were determined from PCR screening. No biochemically confirmed *Salmonellae* were detected. Pathogenic *Listeria* spp. were recovered from strawberry and soil samples on one farm by enrichments in 2007. *E. coli* in excess of drinking water quality limits were recovered from 7 of 17 and 11 of 28 samples in 2006 and 2007, respectively.

Significance: Interview and observation data indicate that both organic and conventional growers are aware of the need for clean irrigation water for fresh produce. Most avoid direct contact of all commodities with animal manure. For drip irrigated crops, growers often used surface water, but were aware of the need to avoid contact on fruits.

P1-30 Comparison of Moist Heat Inactivation Rates of *Salmonella* Enteritidis and *Pediococcus* spp. NRRL B-2354 on Whole Almonds under Commercial Plant Conditions

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Introduction: Outbreaks of *Salmonella enterica* serotype Enteritidis infections have been associated with raw almonds. *Pediococcus* spp. NRRL B-2354 was previously shown to have dry heat inactivation characteristics comparable to *Salmonella* Enteritidis Phage Type 30.

Purpose: The objective of this study was to evaluate the thermal inactivation rate of a non-pathogenic surrogate culture—*Pediococcus* spp. NRRL B—2354 for *Salmonella* Enteritidis Type 30 on whole almonds treated with moist heat, using a commercial steam pasteurizer at different production rates.

Methods: Whole almonds were inoculated with each culture at greater than 8 log CFU/g. The inoculated samples were exposed to moist heat at 210°F in the pasteurizer at three different production settings. Almonds were analyzed by the spread plate technique for both organisms, using Tryptic Soy Agar incubated at 35°C for 48 h.

Results: Log reductions were obtained by subtracting log of survivors of treated samples from log of counts of untreated inoculated controls. Under the test conditions, when exposed to the moist heat treatment, *Pediococcus* spp. NRRL B-2354 showed less heat resistance than *S.* Enteritidis PT30.

Significance: Data showed that overall reduction values for both organisms were greatly affected by production rates (lbs/h) and vibration levels (RPM). Production conditions should be optimized to achieve a desired level of reduction of *S.* Enteritidis PT30.

P1-31 Inactivation of *Salmonella* Enteritidis PT30 by Low-Energy X-Ray Irradiation on Almonds at Different Water Activities

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Introduction: Recent outbreaks of salmonellosis traced to raw almonds have shown that raw nuts can become contaminated with bacterial pathogens during and after harvest. Irradiation is gaining support as a pasteurization technique that can inactivate pathogens while retaining raw product functionality. However, the efficacy of irradiation partially depends on the surface water activity (a_w) of the product and the type of irradiation.

Purpose: The purpose of this study was to quantify and model inactivation of *Salmonella* Enteritidis PT30 (SE PT30) by low-energy x-ray (70 keV) irradiation on almonds at different surface a_w values.

Methods: Raw almonds were surface-inoculated with SE PT30 to a final cell concentration of ~8.5 log CFU/g and dried for 24 h at 24°C. Subsequently, the dried samples were transferred to a dessicator containing saturated solutions of potassium acetate (CH_3COOK) or sodium chloride (NaCl) to obtain surface a_w values of 0.23 and 0.65, respectively. The almonds were then irradiated, using a low-energy x-ray food irradiator (Rayfresh Foods Inc., Ann Arbor, MI) to estimate the efficacy at different a_w values. Survivor curves were plotted against surface x-ray dose as measured using with radiochromic film dosimetry. Radiation D_{10}-values (the cumulative dose needed to inactivate 90% of the target organisms) were then calculated and compared using ANOVA.

Results: SE PT30 was significantly ($P < 0.05$) more resistant to low-energy x-ray irradiation at a_w 0.65 (D10 ~0.34 kGy) compared to a_w 0.23 (D_{10} ~0.26 kGy).

Significance: Inactivation of SE PT30 on almonds needs to be modeled as a function of a_w and irradiation dose before low-energy x-ray can become a viable microbial reduction strategy for this industry. Although almonds were used in this study, these findings will likely be useful for assessing the efficacy of low-energy x-ray irradiation in other low a_w foods, including other nuts, grains and flours.

P1-32 DSC The Survival of *Salmonella* Enteritidis on Walnuts during and after Walnut Hulling

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Introduction: Salmonellosis has been associated with consumption of almonds, peanuts and peanut butter. Although survival of *Salmonella* in these products has been well studied, very little is known about the behavior of this organism in walnuts.

Purpose: To determine the survival of *Salmonella* in walnut hulling waste and on hulled walnut shells.

Methods: Hull waste materials were collected from a local walnut huller and walnuts were obtained from a local processor. Because waste materials from hullers are often applied to walnut orchards, moist hull pieces, hull slurry (ground hulls and water), and hull wash water were inoculated with *Salmonella* Enteritidis Phage Type 30 at 9–10 log CFU/g or ml and held at 23°C for 4 weeks. Whole in-shell walnuts were inoculated at 6, 8, and 10 log CFU/nut and held at 23°C for up to 12 weeks. *Salmonella* was recovered by vigorous shaking in 0.1% peptone and appropriate dilutions were plated onto Tryptic Soy and Bismuth Sulfite Agar.

Results: Salmonella levels in wash water, hulls, and hull slurry steadily but slowly declined by 3 log CFU/g or ml over 4 weeks at 23°C. The survival of *Salmonella* on walnut shells was significantly impacted by initial inoculum concentration. When inoculated at 10, 8, and 6 log CFU/nut, initial reductions during drying were 0.7, 1.5, and 2.4 log CFU/nut, respectively; additional reductions of 0.9, 2.8, and 2.6 log CFU/nut, respectively, were observed over the first 4 weeks of storage. After 12 weeks of storage *Salmonella* was not detected (< 1.7 CFU/nut) in walnuts inoculated at 8 or 6 log CFU/nut but remained at log 7 CFU/nut on samples inoculated at 10 log CFU/nut.

Significance: Although hull waste materials may provide a source of contamination of walnuts, *Salmonella* does not survive well on walnut shells even when initial contamination levels are relatively high.

P1-33 DSC Use of the Weibull Model to Evaluate the Impact of Storage Time and Temperature on Thermal Inactivation of *Salmonella* Enteritidis PT 30 on Oil Roasted Almonds

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Introduction: Survival curves of *Salmonella* during heating of almonds are not log linear. The Weibull model is often used to describe non-linear thermal inactivation of microbial cells.

Purpose: To quantify nonlinear survival of *Salmonella* after immersion in hot oil, using the Weibull model.

Methods: Whole almonds inoculated with *Salmonella* Enteritidis PT 30 (8.7 log CFU/g) were stored at 4 and 23°C. Almonds (50 g) were sampled at 1, 12, 24, 37, and 48 weeks, submerged in 121°C oil, heated for 0.5, 1.0, 1.5, 2.0, or 2.5 min (six samples per heating time), drained for 10 s, transferred to 100 ml of cold (4°C) Tryptic Soy Broth, and stomached for 2 min. Appropriate dilutions were plated onto bismuth sulfite agar and incubated at 37°C for 48 h. Data were modeled with the Weibull equation, $S(t) = \exp(- (t/\alpha)^{\beta})$, where S is the number of survivors made dimensionless with the initial population, α and β are the scale and shape parameters, respectively, and $(1/\alpha)^{\beta}$ is the rate constant.

Results: Populations of *Salmonella* remained stable during storage at 4°C but declined significantly ($P < 0.05$) over 48 weeks by 2.1 log CFU/g when stored at 23°C. Each survival curve generated upon heating in oil was biphasic and well described by the Weibull expression. The shape factor β was < 1 (indicative of tailing) at both storage temperatures and at all storage times. The survivor curves did not differ when almonds were stored at 4°C ($\beta = 0.31 \pm 0.03$, rate constant = 11.0 ± 0.5 $min^{-\beta}$). Reductions of 4- or 5-log were consistently achieved after heating for 0.58 ± 0.08 or 1.18 ± 0.17 min, respectively. In contrast, at 23°C differences in β and the rate constant were observed at 1 week of storage and beyond. Reductions of 4 or 5-log reduction were achieved after heating for 1.16 ± 0.36 and 2.06 ± 0.57 min, respectively, indicating that populations surviving storage were more resistant to heat.

Significance: The Weibull model can be used to analyze nonlinear survival of *Salmonella* on oil roasted almonds.

P1-34 Microbiological Evaluation of Step-by-Step Process in Seed Sprouting

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Introduction: With the recent shift in consumer life style towards 'Healthy living and healthier foods,' the consumption of raw sprouts, mostly in salads and sandwiches, has increased in popularity. However, little was known about changes in the microbial population of the sprouting process in Korea.

Purpose: The purpose of this study was to evaluate the microbial quality of the step-by-step process in seed sprouting.

Methods: Microbial analyses of five domestic seed samples (cabbage, radish, rape, red radish, vitamin) and the five imported seed samples (alfalfa, broccoli, clover, red kohlrabi, red radish) included aerobic plate count (APC) and coliform count, as well as detection of *Bacillus cereus*, *E. coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Vibrio parahaemolyticus*.

Results: Bacteriological analysis showed that the seeds had an APC ranging from 10^3–10^5/g and coliforms counts ranging from 10^1–10^3/g. Samples from seed germination till sprouting contained high numbers of microbial flora (10^6–10^8/g), including coliforms (10^5–10^7/g). *E. coli* was detected in domestic radish, rape, and red radish seed during sprouting.

Significance: This study makes a scientific contribution to the field of food safety of significant interest for sprout consumers and producers. There is a need to prepare a safety regulation program and conduct effective decontamination processes to improve the microbial safety of commercial sprouts.

P1-35 Detection and Recovery of *Escherichia coli* O157:H7 in Artificially Contaminated Alfalfa Sprouts by PATHATRIX Immunomagnetic Separation, Real-Time PCR and Cultural Methods

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Introduction: Escherichia coli O157:H7 has been responsible for a number of foodborne disease outbreaks associated with alfalfa sprouts. However, it can be challenging to isolate this organism from sprouts because of the presence of high background counts associated with the fresh product.

Purpose: To improve upon the current methods for detection of *E. coli* O157:H7, immunomagnetic separation (IMS) and real-time PCR, in combination with different selective enrichment methods, were evaluated. Because of excessive growth of non-target microorganisms on the selective agars used for isolation, post-enrichment processing by IMS beads including dilution (1:25) and post-capture washing with Tween 20 prior to plating were investigated.

Methods: Two enrichment media (modified Buffered Peptone Water with pyruvate, mBPWp, and EHEC Enrichment Broth, EEB) were compared after 24 h for the detection of *E. coli* O157:H7 in artificially contaminated alfalfa sprouts. Both treatments also employed re-circulating IMS by PATHATRIX to improve detection by real-time PCR (*stx*1, *stx*2, O157:H7 uidA mutation targets) and recovery on Levine's EMB, Tellurite-Cefixime Sorbitol MacConkey, and R&F Chromogenic Agar. Additional experiments using mBPWp were conducted in alfalfa sprouts inoculated at low (~0.1 CFU/g) levels of *E. coli* O157:H7. Samples were stomached for 2 min in mBPWp with selective agents and incubated at 42°C for 5 h and 24 h. The processed IMS beads, 1:25 dilution of mBPWp prior to IMS, 3X Tween20 rinsed beads, and combination 1:25 dilution and 3X rinsed beads were analyzed by real-time PCR and plated to selective agars.

Results: After 24 h, the mBPWp greatly improved detection and recovery of *E. coli* O157 over EEB. PATHATRIX IMS treated enrichments increased recovery of *E. coli* O157:H7 over non-IMS treatments.

Significance: At low levels of *E. coli* O157:H7 inoculation, neither dilution of mBPWp (1:25) before immunoconcentration nor washing of the beads nor a combination of dilution and washing after collection improved cultural detection.

P1-36 DSC Reduction of Salmonellae Inoculated onto Alfalfa Sprouts' Surfaces by Gaseous Chlorine Dioxide

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Introduction: Salmonella has been the primary pathogen of concern in fresh, raw sprouts, (especially alfalfa sprouts) since the first large international outbreak of salmonellosis in 1995. Despite the research on physical and chemical methods and the manufacturer-recommendations of the US Food and Drug Administration (FDA), 26 outbreaks of alfalfa sprouts and more than 1636 cases of illnesses have been reported by the Centers for Disease Control and Prevention (CDC) from 1995 to 2004. This indicates that the current practices are not adequate to prevent salmonellosis from the consumption of fresh, raw sprouts.

Purpose: Our objective was to investigate the reduction of salmonellae on sprout surfaces by chlorine dioxide (ClO_2) gas treatments. Our goal was to achieve a 5-log reduction of salmonellae on the sprout surfaces, consistent with the recommendations of the United States Department of Agriculture — National Advisory Committee on Microbiological Criteria for Foods (USDA-NACMCF).

Methods: A mixed culture of 3 *Salmonella* strains (*Salmonella* Mbandaka, *Salmonella* Newport and *Salmonella* Stanley), recovered from alfalfa sprouts outbreaks was spot inoculated onto sprouts surfaces. The sprouts were then treated with 3.0 and 5.0 mg/l ClO_2 gas for 6–20 min at 22°C and 90% relative humidity. Surviving bacteria on treated areas were resuscitated and enumerated, using a non-selective medium (Trypticase™ Soy Agar) with selective medium overlay (Xylose Lysine Deoxycholate).

Results: A greater than 3 log reduction of *Salmonella* per gram of sprouts was observed with 3.0 or 5.0 mg/l ClO_2 gas treatment after 20 min. Similar results were obtained in a miniaturized industrial sized ClO_2 gas tunnel system, using *H. alvei* as a surrogate organism, to validate the laboratory studies.

Significance: These results demonstrate that treatment of sprouts with ClO_2 gas has the potential to be successfully employed for the treatment of contaminated alfalfa sprouts to reduce the risk of *Salmonella*-related outbreaks.

P1-37 Efficacy of Chlorine Dioxide Gas and Various Freezing Rates on the Microbiological Quality of Frozen Blueberries

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Introduction: Blueberries are prone to contamination with microbial growth during bulk freezing, negatively impacting quality and marketability.

Purpose: The combined impact of ClO_2 gassing and freezing rate on the microbiological quality of frozen blueberries was examined.

Methods: Thirty lugs of blueberries (~ 9.1 kg/lug) were palletized (5 lugs/level × 6 levels/pallet). In each of four 12 h trials conducted at ~12–14°C, one pallet was tarped with a plastic sheet, sealed, and exposed to ClO_2 gas (0.13 mg/g fruit) using three 3-kg sachets (ICA TriNova), with one pallet remaining untarped (control). Before and after commercial processing, 50-g samples of gassed and un-gassed blueberries were quantitatively examined for mesophilic aerobic bacteria (MAB), yeasts, and molds. Additional 50-g samples were placed at -20°C in different types of insulated containers so as to freeze after 3 h (quick), 2 days (intermediate) and 5 days (slow) and sampled during freezing and 3 months of frozen storage.

Results: Microbial populations decreased significantly ($P < 0.05$) in ClO_2-gassed compared to ungassed blueberries, with the least amount of re-growth seen in quick frozen berries. Regardless of freezing rate, significant ($P < 0.05$) reductions in MAB (> 1 log CFU/g) occurred between the ClO_2-treated berries and un-gassed controls. Significantly ($P < 0.05$) lower yeast populations (> 0.62 log CFU/g) were seen in quick and intermediate frozen gassed compared to un-gassed blueberries. For quick-frozen blueberries, significant ($P < 0.05$) reductions in molds (~0.56 log CFU/g) were seen between ClO_2-treated berries and the non-gassed controls, with MAB, yeast and mold populations reduced by 2.9, 2.3 and 1.7 log CFU/g, respectively, for ClO_2-gassed quick frozen and ungassed slow frozen blueberries.

Significance: Based on these findings, ClO_2 gas treatment followed by quick freezing affords one means of meeting the current microbial standards for blueberries being imposed by buyers.

P1-38 Impact of Harvesting and Handling on Bell Pepper Contamination with Viral Pathogens

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Introduction: An ill and asymptomatic person may shed high levels of viruses in their feces. Poor hand washing technique makes hands a potential means of transferring human enteric viruses from workers to fresh produce.

Purpose: The aim of the study was to evaluate the impact of harvesting and handling on bell pepper contamination with Hepatitis A virus and Norovirus.

Methods: The study was conducted in Sinaloa, a northwestern state of México, from January to March 2007. Samples from 97 workers' (harvesters, classifiers and packers) hands were collected before and after 3 h of labors. Also, twenty green bell peppers were collected in the field and 20 at the packinghouse. For each hand and produce sample, a PCR-based method were used to detect HAV and NV. Additionally, coliforms and *E. coli* were detected by chromogenic agar.

Results: HAV was absent from either workers' hands or bell peppers. On the contrary, NV was found in 30.9% (30/97) and 23.7% (23/90) of workers' hands before and after three hours of labor, respectively. NV was also detected in 45% (9/20) and 30% (6/20) of bell peppers collected at field and packinghouse operations, respectively. Binary logistic regression analysis showed no correlation between coliforms and *E. coli* presence and NV ($P > 0.05$). Phylogenetic analysis showed that the NV isolated strains belonged to GII/4, and one new genotype cluster (GII/new).

Significance: Results showed that the actual microbial indicators prove not to be useful for predicting viral contamination. Also, the importance of rapid methods for concentrating and detecting viral pathogens should be implemented as a tool for tracking viral origins. Finally, this study showed that field and packinghouse operations might be impacted by human fecal pollution and thus, total adherence to good agricultural practices is suggested to minimize fresh produce viral contamination.

P1-39 Assessing Microbiological Quality of Produce from a Gleaning Project in Nashville, Tennessee

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Introduction: To divert distressed fruits and vegetables out of the waste stream and potentially improve the health and well-being of limited-resource individuals, projects such as the Good Food for Good People Food Recovery Program are collecting foods that might otherwise be discarded, salvaging the edible products, and distributing them to potential consumers. There is little information concerning microbiological quality of the recovered foods.

Purpose: The purpose of this study was to evaluate the overall microbiological quality of produce recovered through the gleaning project and compare it to products purchased the same day that were deemed sellable.

Methods: A total of 138 samples, including tomatoes, bell peppers, cucumbers, and yellow squashes, from the gleaning project and 67 control samples from local grocers were collected. Microbial loads of samples were determined by aerobic plate count (APC), and *Enterobacteriaceae* count (EC).

Results: The averages of microbial load for gleaned tomatoes were 1.9×10^6 CFU/g for APC and 8.9×10^5 CFU/g for EC, which were about one $\log_{10}$ higher than the averages of control samples. There was no significant difference between other produce from gleaning project and controls ($P > 0.05$). The averages of microbial load for gleaned bell peppers were 6.5×10^5 CFU/g for APC and 1.1×10^5 CFU/g for EC, the averages for gleaned cucumbers were 6.6×10^5 CFU/g for APC and 1.2×10^4 CFU/g for EC, and the averages

for gleaned yellow squashes were 1.6×10^5 CFU/g for APC and 8.4×10^4 CFU/g for EC, respectively. *Enterobacter sakazakii, Enterobacter cloacae* and *Klebsiella pneumoniae* were found in some of the recovered and control samples.

Significance: The microbiological quality of most recovered produce at the point of distribution was comparable to that of produce purchased from grocers. Because of their perishable nature, recovered tomatoes were of marginal quality.

P1-40 DSC Washing Effect of Sodium Hypochlorite with 5% Acetic Acid and Acidified Sodium Chlorite on Reduction in Population of Foodborne Pathogens on Fresh Produce

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Introduction: A number of foodborne outbreaks associated with fresh produce have caused some concerns to the industry and consumers. Washing is one of the most important control measures implemented to reduce the risk of product contamination. However, the equal effectiveness of sanitizers is questionable depending on the types of pathogens and produce.

Purpose: The objective of this study was to investigate washing effect of sodium hypochlorite (Cl), sodium hypochlorite with 5% acetic acid and acid sodium chlorite (ASC) on the reduction of pathogens inoculated onto fresh produce.

Methods: Nalidixic acid-resistant strains, log phase or stationary phase of *S.* Typhimurium and *L. monocytogenes* were used for inoculation of produce. 10 g of cherry tomatoes, cucumbers or carrots were washed for 5 or 10 min in Cl, or in Cl with 5% acetic acid (100 or 200 ppm) or in 500 ppm ASC.

Results: Overall, the sanitizer reduced *S.* Typhimurium populations on produce more significantly than those of *L. monocytogenes*, regardless of the type of produce. 5% acetic acid in combination with 200 ppm Cl was the most effective washing treatment among the sanitizing agents tested. The Cl with 5% acetic acid was significantly ($P < 0.05$) more effective than ASC in reduction of *S.* Typhimurium, but not in the reduction of *L. monocytogenes*. In addition, washing treatments were most effective on cucumbers, followed by carrots and tomatoes. Populations of *S.* Typhimurium inoculated onto cucumber were reduced about 6 log cycles after washing with 200 ppm Cl with 5% acetic acid. *S.* Typhimurium cells in stationary phase were reduced more than those in log phase.

Significance: These results indicate that the efficacy of sanitizer varies according to the type of produce and the kinds of pathogens on fresh produce. Thus, guidelines on the usage of sanitizers are needed to increase the effectiveness of sanitizer.

P1-41 Evaluation of Commercial Test Kits and Tangential Flow Filtration for Detection of *Salmonella* in Spent Mung Bean Irrigation Water

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Introduction: Microbiological testing of spent irrigation water has been recommended as part of an overall strategy to enhance the safety of sprouts. Many rapid tests have been developed in recent years and a number of them have been certified by the AOAC. Whether these tests are capable of detecting low levels of *Salmonella* in spent sprout irrigation water remains to be determined. The extra cost associated with microbial testing of sprouts has hindered the full implementation of this recommendation by the industry. Sample pooling has been proposed to lower the cost. There is a need to develop sample preparation methods that would allow concentration of the entire pooled sample for analysis by a single test.

Purpose: The first part of this research was to evaluate the performance of a number of commercial test kits for detection of *Salmonella* in spiked mung bean irrigation water. The second part of this research was to examine the feasibility of a tangential flow filtration system (TFF) for concentration of *Salmonella* from large volumes of mung bean water.

Methods: GENE-TRAK *Salmonella* DLP assay, BAX for Screening *Salmonella*, Reveal for *Salmonella*, TECRA *Salmonella* ULTIMA and Assurance Gold *Salmonella* EIA were evaluated, along with the standard method described in the FDA Bacteriological Analytical Manual (BAM). Twenty-five ml of mung bean water collected at 48 h of sprouting was inoculated with 1, 10 or 100 CFU of *Salmonella* cocktail and was subjected to the enrichment and assay protocols recommended by each method. To examine the feasibility of TFF for concentration of *Salmonella* from large volumes of mung bean water, 10 log of mung bean water were inoculated with 0, 1, 10, of 100 CFU of *Salmonella* and concentrated 100-fold using the TFF system. The presence of *Salmonella* in the concentrated samples was determined with the BAM method.

Results: It was found that all test kits were able to detect the presence of *Salmonella* at a level of 1 CFU/25 ml. With the TFF, it was found that as few as 1 CFU of *Salmonella* could be recovered from 10 log of sprout water.

Significance: These results suggested that additional commercial test kits other than those recommended by the FDA are also useful for detection of *Salmonella* in spent mung bean irrigation water, and that incorporation of TFF could allow testing of 10 L of sprout water with a single test and improve the detection limit of existing methods to 1 CFU/10 log.

P1-42 Dump Tank Water Sanitation Technologies

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Introduction: Many horticultural producers use water as a method to remove field heat and/or to clean produce. Water is a well-known vector for microbial contamination, and a number of trace back investigations have suggested that poor dump tank water quality contributed to outbreaks in fresh fruit and vegetables. To avoid this, producers typically treat their dump tank water with sodium or calcium hypochlorite; however, these treatments are corrosive, have worker safety issues, and are not in accordance with organic production standards. In addition, the efficacy of these treatments is reduced by the presence of organic matter (OM), which can be present in high concentrations depending on the type of produce.

Purpose: As a result, seven water treatment systems, calcium hypochlorite, sodium hypochlorite, chlorine dioxide, ultra violet radiation, ozone and two different peroxyacetic acid treatments, were evaluated for efficacy, worker safety, ease of use and disposal, cost and changes in produce quality as a result of their use.

Methods: The research was conducted in a research pilot plant, and designed as 2 × 2 factorial experiment with high and low levels of OM and *Escherichia coli* for each of the seven treatments. Asparagus, lettuce and peaches were assessed for color and textural changes against all seven treatments in a randomized complete block design with four replications.

Results: All treatments exhibited at least a 94% reduction in *E. coli*, with an interaction between the *E. coli* and OM factors. Each treatment varies significantly in terms of cost, worker safety and ease of use. Color and textural changes were detected and the degree of change was dependent on the treatment.

Significance: This research provides producers practical information enabling them to make informed decisions implementing water treatment technology, which enhances food safety while meeting their production needs.

P1-43 Transfer Prevalence of *Escherichia coli* O157:H7 from Soil, Water, and Manure Contaminated with Low Numbers of the Pathogen to Lettuce Plants of Varying Age

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Introduction: The continued large scale *E. coli* O157:H7 outbreaks associated with leafy greens suggests data gaps exist at all levels from the farm to the consumer with respect to microbial safety. The sources of contamination of leafy greens remain unclear, but it is evident that contaminated water, soil amendments, and wildlife likely contribute.

Purpose: The objective of the present study was to determine transfer, prevalence, and persistence of low numbers of *E. coli* O157:H7 from soil, water, and feces to lettuce.

Methods: Lettuce plants, young (12 days old) or aged (30 or 40 days old), were exposed to soil, irrigation water, or cow manure contaminated with 10^1, 10^2, 10^3, or 10^4 CFU *E. coli* O157:H7/g or ml. Harvested plants were processed to determine *E. coli* O157:H7 associated with the entire plant or at internal locations. Young plants (12 days) were harvested at 1, 10, 20, and 30 days post exposure. Aged plants were harvested at 45 days of age.

Results: No samples were positive for *E. coli* O157:H7 following direct plating of serial dilutions. Enrichment of samples demonstrated 9% (26 of 288) of plants had internalized *E. coli* O157:H7 regardless of challenge dose; external portions of young plants were contaminated for up to 30 days post-exposure. Aged plants exposed to contaminated soil (10^4 CFU/g) demonstrated *E. coli* O157:H7 populations up to 3.4 log CFU/g. Aged plants exposed to other media (water, manure) were culture positive only following enrichment, but were positive 15 days after initial exposure to the pathogen. This was the situation whether samples were tested for internal or internal/external target bacteria and regardless of initial challenge level.

Significance: Although experiments were not conducted under true environmental conditions, the research indicates that lettuce exposed to and grown in the presence of low numbers of *E. coli* O157:H7 may become contaminated and present a human health risk.

P1-44 Effects of Time and Sanitizer Concentration in Produce Wash Water on Cross Contamination of *Salmonella* and *Escherichia coli* O157:H7

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Introduction: Fresh produce is washed with water that may contain sanitizing agents. Does washing reduce or increase cross contamination of fresh produce with *Salmonella* and *E. coli* O157:H7?

Purpose: This study determined cross contamination of fresh produce with *Salmonella* and *E. coli* O157:H7 as influenced by amount of time produce is washed in water with and without sanitizers.

Methods: Salmonella was inoculated onto tomatoes and cantaloupes. *E. coli* O157:H7 was inoculated onto lettuce pieces. Wash treatments included water, water with 50 or 100 ppm chlorine (pH 6.0–7.5), or water with 80 ppm peroxyacetic acid. Inoculated tomatoes were placed in the wash tank with uninoculated tomatoes and continually agitated. Uninoculated tomatoes were removed, placed in neutralizing broth, and sampled for *Salmonella*. Cantaloupes were treated in a similar manner. Pieces of chopped lettuce were placed in the tank with pieces of uninoculated lettuce. After 45 sec, uninoculated pieces were removed, placed in neutralizing broth, and sampled for *E. coli* O157:H7. Three replications were done.

Results: By 2 min, most tomatoes without sanitizer became contaminated with *Salmonella*; by 4 min all were positive. When exposed to 50 ppm chlorine, 11–16% of tomatoes were positive at all sampling times, while exposure to 100 ppm chlorine prevented contamination of the tomatoes. Approximately 11% of tomatoes exposed to peroxyacetic acid were contaminated with *Salmonella* after 2 min, but after 4 min, none were positive. For cantaloupes without sanitizer, all were positive for *Salmonella*. Cross contamination of cantaloupes did not occur with the peroxyacetic acid treatment, while chlorine treatments were less effective (28–78% positive). For lettuce, chlorine exposure for 45 s was effective in preventing *E. coli* O157:H7 cross contamin-ation. Peroxyacetic acid was less effective.

Significance: Effectiveness of sanitizing agents and concentrations varied depending on type of produce used. This data could be used when evaluating process control points.

P1-45 DSC Microbiological Quality of Surface Water Used for Irrigation of Fresh Vegetable in Mpumalanga, South Africa

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Introduction: The microbiological quality of a body of water behind a dam and two rivers that feed in Mpumalanga, South Africa and is used for irrigation of fresh vegetables was investigated with respect to aerobic colony counts, aerobic sporeformers, anaerobic sporeformers and presence of fecal coliforms, *E. coli*, *Salmonella*, *Listeria*, intestinal enterococci and *Staphylococcus aureus*. Other physico-chemical parameters determined in the irrigation water were pH, turbidity and COD.

Purpose: The objective of the work is to determine the type and number of bacterial pathogens in the surface water since it is used for irrigation of fresh vegetables that are eaten raw. Furthermore, consumption of vegetables contaminated with foodborne pathogens by South Africans might lead to outbreaks of foodborne illnesses, and a large proportion of the citizens have compromised immune system diseases such as HIV and tuberculosis.

Methods: Gas-forming organisms, coliforms, fecal coliforms and *E. coli* were recovered using MPN (Most Probable Number) while other bacterial pathogens were recovered with conventional method (enrichment media and selective media). The pH was measured with a 211 Microprocessor pH meter (Hanna Instruments Inc., Woonsocket, RI, USA), while turbidity was determined with a H1 93703 Microprocessor turbidity meter (Hanna Instruments Inc., Woonsocket, RI, USA). Chemical Oxygen Demand (COD) was measured with the closed reflux colorimetric method, as described in standard methods (APHA, 2001)

Results: The average fecal coliforms for the three points where potential irrigation water samples were collected was higher than 1600 MPN/100 ml. This level is higher than the standard approved by the WHO for irrigation water. A high level of *E. coli* was also recovered from the three surface water samples. *Salmonella* and *Staphylococcus* were not recovered from all three irrigation water samples but presumptive *Listeria* was isolated from one of the three surface water samples. COD and turbidity of the three water samples were higher than WHO and SA water guidelines, although the pH met the standard.

Significance: The high level of fecal coliform and *E. coli* recovered from the three surface water samples and the presence of *Listeria* in one surface water sample clearly shows that fresh vegetables from this area may constitute a health risk for the consumers.

P1-46 Effect of Coffee Cherries Storage after Harvest before the Beginning of Drying on Contamination by Fungi and the Relationship to Ochratoxin A Production

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Introduction: The main mycotoxin found in coffee is Ochratoxin A produced by molds of the genus *Aspergillus* in tropical regions and by molds of the genus *Penicillium* in moderately cold regions. This mycotoxin has been classified as a possible human carcinogen. Its occurrence can be due to environmental conditions and badly controlled post-harvesting. Avoiding this contamination is a must.

Purpose: The present study was carried out to evaluate the influence of the storage of coffee cherries after harvest and before the beginning of drying on fungal growth and kinetics of Ochratoxin A production in order to contribute to the identification of good post-harvest practices to prevent development of this mycotoxin.

Methods: In this study, Robusta (*Coffea canephora P.*) coffee cherries from Côte d'Ivoire were used. Ripe coffee cherries after harvest were divided into four lots of 60 kg each. The drying of one of the lots was started the day of harvest. For the three others lots, the drying was started the second, the fourth and the sixth day after harvest. The drying was carried out on a 3 m^2 area of concrete which is the most commonly used drying area type in the Ivory Coast. Coffee cherries were covered at night and if there was rain during the day in order to avoid rewetting. Three samples of 500 g from each drying area were taken everyday during drying until the moisture content of coffee cherries reached between 11 and 12%. The research of fungi inside the coffee cherries was carried out by sterilizing parchment coffee in 5% sodium hypochlorite for 1 min and washed three times with sterile distilled water. Parchment coffee sterilized was then plated onto the medium (Sabouraud Chloramphenilcol Agar) into the Petri dishes (ten per plate). Petri dishes were incubated at 30°C for 7 days. The results were expressed as the percentage of samples containing infected beans. All the moulds isolated were subcultured on Czapeck-Dox Agar and identification was performed according to the classification scheme of Raper & Fennel (1965) and Samson et al. (1995). The test for OTA production by isolated fungi was carried out by using the method of Bragulat et al. (2001). Analysis of OTA in coffee was carried out by using the method of Pittet et al. (1996). A total of 25 g finely ground green coffee were extracted with a solution of methanol 3% sodium bicarbonate (50:50). The OTA was quantified by reverse-phase using a fluorescence detector FL3000. The HPLC column was a C18 Sorbox SB-48 (5µm 4.6 times 150 mm) (Agitent, USA). A total of 80 ml of each sample was injected. The mobile phase consisted of acetonitrile/acetic acid 0.2% (41:59). The flow rate was 1ml/min^{-1}. The retention time for the detection of OTA was around 14 min. The detection limit was 0.3 mg/kg^{-1} OTA.

Results: The results obtained show that for coffee cherries for which drying was started the day of harvest, only 5% of the samples analyzed contained infected beans, while for those for which drying was started the fourth and the sixth day after harvest, all the samples analyzed contained infected beans. The identification of fungi isolated shows that the most common species found was *A. niger* (42.19–100%). The test for Ochratoxin A production by isolated fungi shows that only strains of *A. niger*, *A. carbonarius* and *A. ochraceus* are Ochratoxin A producers. The evaluation of Ochratoxin A content shows that coffee cherries most contaminated by this mycotoxin are those for which drying was started the sixth day after harvest, with levels ranging from 51.27 to 1517.12 µg/kg of green coffee.

Significance: The storage of coffee cherries after harvest before the beginning of the drying is most likely one of the main parameters contributing to Ochratoxin A production. Thus, the prevention and the reduction of Ochratoxin A production in coffee can be achieved by starting the drying the day of harvest.

P1-47 DSC Determination of Aflatoxin Levels in Herbal Medicines by ELISA-HPLC-LC/MS/MS

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Introduction: Although many methods have been developed to detect aflatoxins in herbal medicines, most detection methods have low sensitivity because of the complex chemical composition of herbal medicines.

Purpose: Objective of this study was to establish an improved method of analysis to detect aflatoxins (AFB1, AFB2, AFG1, AFG2), using a combination of methods of enzyme-linked immumosorbent assay (ELISA), high performance liquid chromatography (HPLC), and liquid chromatography combined with tandem mass spectrometry (LC/MS/MS).

Methods: Seventy herbal medicine products (10 samples per product) were purchased from retail markets. Ground medicine samples were extracted with 60% methanol followed by filtration, and the extract was diluted in phosphate buffered saline. Then, the diluent was added to microtiter plates coated with monoclonal antibodies to screen for possible aflatoxin-positive samples, using ELISA. The positive samples were reextracted with 70% methanol and cleaned through an immunoaffinity column for determination of aflatoxin concentrations by HPLC equipped with Coring cell for derivatization. Aflatoxin positive samples from HPLC were confirmed consecutively by LC/MS/MS. For valldation of this procedure, recovery, standard deviation, and detection and quantification limits of this method were determined with samples artificially contaminated with AFB1.

Results: Standard deviations (ELISA: 0.89–8.52; HPLC: 4.06–6.31) and recovery (ELISA: 84.30–89.99; HPLC: 81.55–102.67%) of the method were acceptable, and limits of detection and quantification for AFB1 were 0.05–0.1 and 0.4–1.0 ng/g, respectively. ELISA identified 58 aflatoxin positive samples (8.29%) of 700 samples. Of the 58 samples, HPLC system identified 18 aflatoxin positive samples at 2.07–156.07 ng/g. LC/MS/MS analysis confirmed the 18 samples were aflatoxin positive, and aflatoxins were eluted at 10.1 (AFB1), 7.6 (AFB2), 8.6 (AFG1), and 6.4 (AFG2) min.

Significance: Use of ELISA for rapid screening, HPLC for quantification and LC/MS/MS for confirmation may be rapid, accurate, and cost-effective in detecting aflatoxin in herbal medicines.

P1-48 Monitoring of Ochratoxin A in Alcoholic Beverages

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Introduction: Ochratoxin A produced by several *Aspergillus* and *Penicillium* shows carcinogenic, immunosuppressive, nephrotoxic, and teratogenic properties. Ochratoxin A has been detected in fermented alcoholic beverages such as wine, beer, fruit wine, medicinal wine and makgoli. It is necessary to monitor Ochratoxin A in alcoholic beverages to ensure their safety.

Purpose: The purpose of this study is to determine Ochratoxin A in alcoholic beverages to accumulate more information about their contamination and to assess the risk of Ochratoxin A to public health.

Methods: Samples used were 177 of wine imported from 5 major exporting-countries, 106 of imported and domestic beer, 74 of fruit fermented alcoholic beverages and 74 of medicinal alcoholic beverages from regions in Korea. The analytical methods for Ochratoxin A reported in journals, including AOAC, were investigated and those for each beverage were selected and modified. The risk to public health by Ochratoxin A was assessed by estimating the portable daily intake (PDI) and comparing it with the tolerable daily intake (TDI) proposed by EU.

Results: Wine and beer were analyzed by the AOAC method. Makgoli and fermented alcoholic beverages were analyzed by methods reported in journals. LOD, LOQ, and recovery in alcoholic beverages were from 0.0024 ng/ml to 0.047 ng/ml, 0.0048 ng/ml to 0.094 ng/ml, and 89% to 97%. Ochratoxin A was detected in 17% of 177 wine samples, 23.6% of 106 beer, 14.9% of 74 makgoli, and 10.1% of 74 fermented alcoholic beverages. The average levels of Ochratoxin A were 0.039 ng/ml in wine, 0.010 ng/ml in beer, 0.57 ng/ml in makgoli, and 0.27 ng/ml in fermented alcoholic beverages.

Significance: Since the PDI values of Ochratoxin A was lower than the TDI value, the health risk associated with Ochratoxin A in alcoholic beverages was not significant. However, investigating the PDI value of Ochratoxin A in age categories, the age group from 50 to 64 consumed Ochratoxin A at 4.7 times the average intake in makgoli. Therefore, a sustainable survey of Ochratoxin A in makgoli will be required.

P1-49 Determination of Patulin Level in Fruit Juices and Juice Concentrates in Korea

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Introduction: Patulin, a mycotoxin mainly produced by *Penicillium* and *Aspergillus*, is found in various foods. At present, a maximum acceptable level for patulin is established at 50 µg/kg (ppb) in apple juices and apple concentrates in Korea. But patulin may be detected in juices produced with other fruits as well as apple.

Purpose: The purpose of this study was to evaluate patulin levels in various types of fruit juices, including apple juice, and to apply the results to the regulation policy.

Methods: Patulin contamination was analyzed in 520 samples of fruit juices and 50 samples of fruit juice concentrates. A method using HPLC/PDA was applied to quantitatively analyze patulin levels in samples.

Results: The results showed that 3 samples of 520 fruit juices and beverages and 5 samples of 50 fruit juice concentrates were contaminated by patulin, 9.8–8.0 µg/kg, and 4.7 – 18.2 µg/kg respectively. Contaminated samples were produced with apple, orange or pear.

Significance: This result indicates that extending regulatory range of patulin, from apple juice and concentrate in the present to fruit juice, beverage and concentrate in the future, should be considered

P1-50 The Exploratory Data on Furan Content in Processed Foods in the Korean Local Market

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Introduction: Furan is often found in processed food and is known to be formed during cooking and heat treatment. The importance of furan in processed food is seen from animal studies which show that furan is a potential carcinogen to humans. The presence of furan in canned and jarred food is considered a potential carcinogen and a new endocrine disrupter to humans.

Purpose: In spite of furan's low levels in food products and exposure in diet, furan could be a carcinogen of major concern. The objective of this study was to provide quantified information about furan and its potential toxicity to humans by performing exploratory work on furan levels in processed foods.

Methods: One-hundred fifty kinds of food samples, including vegetable jarred/canned foods, processed meat canned foods, canned sea food, and jarred/

canned juices, were purchased according to the best selling list published by *Food Journal* from local markets in Korea. A 1~10 gram test portion of liquid, semi-solid, or solid foods were diluted with water, fortified with internal standard (d4-furan), and sealed in headspace vials. The solid phase micro extraction headspace sampling method followed by gas chromatography/mass spectrometry (GC/MS) analysis was used to detect furan and d4-furan in the sim mode.

Results: The level of furan in processed canned meat (32.16 ppb) was the highest among the samples in this study. Jangjorim, processed beef showed average 85.72 ppb of furan concentration. The furan levels in canned sea food and canned vegetables were 29.40 ppb and 22.86 ppb, respectively. This study may provide data to determine furan levels in processed foods, which eventually may be helpful in preventing potential damage by furan.

Significance: This study may provide data for databasing the furan levels in foods, which eventually may prevent damage by furan.

P1-51 Comparison of Different SPE (Solid-Phase Extraction) Methods for the Analysis of Heterocyclic Amines from Fried Pork Patties

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Introduction: Human populations are exposed to mutagenic or carcinogenic heterocyclic aromatic amines (HCAs) through various food products. These compounds are multi-potential carcinogens. Various extraction and purification methods have been used to analyze the HCAs from various foods. To better assess the contribution of HCAs in human cancer etiology, human intake needs to be accurately analyzed.

Purpose: The purpose of this study was to evaluate four different extraction and purification methods for determination of 15 HCAs in fried pork patties.

Methods: Four different extraction and purification methods were evaluated to determine the HCAs in fried pork patties. Pork patties were cooked in a teflon-coated electric frying pan at 225°C for 10 min per side. HCAs (IQ, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, Tri-MeIQx, PhIP, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, A£\C, MeA£\C, Harman, Norharman) in the fried pork patties were extracted and purified using four different solid-phase extraction methods and were quantified by LC-MS(API-ESI). Recovery by four different extraction and purification methods was evaluated by comparing the HCAs amounts quantified by standard addition method. Validation of extraction and purification method for fried pork patties was determined to establish the accuracy of sample preparation.

Results: The recoveries of HCAs from different solid-phase extraction (SPE) methods were calculated. Scope of recovery values were 35.89 – 68.69% (Polar amine group) and 15.70 – 74.70% (less-polar amine group) in method A. Method D provided recovery values ranging from 14.14% to 68.65% (polar amine group) and from 2.97% to 72.30% (less-polar amine group).

Significance: These results suggest that the modified procedure of Method A and D were the most suitable extraction and purification method for analysis of HCAs from fried pork patties.

P1-52 Monitoring and Risk Assessment of Furan in Processed Foods by Solid Phase Microextraction Gas Chromatography Mass Spectrometry (SPME-GC/MS)

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Introduction: Furan (C_4H_4O) is a volatile compound formed during the Maillard reaction and has been classified recently as a possible human carcinogen (B2 class) by the International Agency for Research on Cancer (IARC). It has been reported to occur in various canned and jarred foods that undergo heat treatment.

Purpose: The aim of this study was to optimize sample preparation according to food matrix for furan analysis, using SPME -GC/MS, and to perform monitoring and risk assessment in various canned and jarred food.

Methods: The 100 processed foods were purchased from local markets. The method is based on optimized headspace sampling of a 20 ml headspace vial containing sample and internal standard (d4-furan). The concentration of furan was determined qualitatively by gas chromatography mass spectrometry (GC/MS).

Results: The optimized amount of sample, adsorption temperature and time of SPME fiber of solid matrix were 0.1g, 50°C, and 20 min, respectively. Those of liquid, semi solid and paste state foods were 5 g, 50°C, and 20 min, respectively. The level of furan in processed meat canned foods (32.16 ng/g) was the highest among the samples in this study. The furan levels in canned seafood, canned vegetable foods, nutrients-fortified drinks, canned soups and bottled sauces were 29.40 ng/g, 22.86 ng/g, 7.28 ng/g, 15.54 ng/g, 22.02 ng/g, respectively. Furan concentrations of baby foods were between 3.43 and 97.21 ng/g. EDE (Estimated Daily Exposure) (116.73 ng/day) and EDI (Estimated Daily Intake) (14.59 ng/kg-bw/day) of furan were the highest in baby foods. However, the EDI of baby foods is still lower than that of US FDA.

Significance: In this study, the optimized analysis method of SPME-GC/MS was suitable for the determination of furan in processed foods. In addition, these data could be a database for risk assessment of furan.

P1-53 Analysis of Heterocyclic Amines in Cooked Meats by High Performance Liquid Chromatography — Mass Spectrometry

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Introduction: Heterocyclic amines (HCAs) formed in heated and cooked meats are known to be mutagenic and carcinogenic compounds. The concentrations and species of HCAs depend on processing methods and conditions. In this study, 15 HCAs were analyzed qualitatively and quantitatively in various foods such as pork meat stock, roasted fatback, roasted bacon, boiled chicken meat, chicken meat stock, chicken breast for salad, and chicken burger patty.

Purpose: The purpose of this study was to analyze HCAs concentration in cooked meats in Korea, using solid-phase extraction (SPE) and high performance liquid chromatography mass-spectrometry (HPLC-MS).

Methods: The seven food samples were cooked in the laboratory or purchased from local markets. To extract and purify the 15 HCAs from matrix in cooked food samples, solid-phase extraction (SPE) methods were used. The concentrations of 15 HCAs were determinated qualitatively by high performance liquid chromatography-mass spectrometry (HPLC-MS).

Results: Norharman, Harman and PhIP were the most frequently detected HCAs in cooked meats. The levels of Norharman, Harman and PhIP in roasted fatback and bacon were higher than those of the other cooked meats. The levels of Norharman in roasted fatback, roasted bacon, and chicken burger patty were 412.7 ng/g, 348.9 ng/g, and 14.1 ng/g, respectively. Harman levels were determined as 990 ng/g, 512 ng/g, 11.9 ng/g, and 9.8 ng/g in roasted fatback, roasted bacon, chicken meat stock, and chicken breast for salad, respectively. Especially, PhIP is classified in group 2B by International Agency for Research on Cancer (IARC). The levels of PhIP in roasted fatback, roasted bacon and chicken burger patty were detected at 258.2 ng/g, 168.2 ng/g and 6.7 ng/g, respectively.

Significance: In this study, the analysis method of SPE coupled with HPLC-MS was suitable for the determination of HCAs in samples. In addition, these analytical results will provide useful in estimating the risk of exposure to HCAs contaminations.

P1-54 Acute Toxicity and Mutagenic Effect of Methanol Extracts of *Cirsium japonicum*

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Introduction: Flavones extracted from *Cirsium japonicum* are widely used as a dietary supplement and functional foods because of their health benefits. However, there is little information on toxicological evaluation of *Cirsium japonicum* extracts for assuring its safe use.

Purpose: The purpose of this study was to evaluate safety of methanol extracts of *Cirsium japonicum* containing flavones by examining acute oral toxicity, bacterial reverse mutation, and chromosome aberration.

Methods: A single dose of 2000 mg of extracts/kg body weight was administrated to both female and male mice. Mutagenicity of extracts was evaluated by a bacterial reverse mutation assay using *Salmonella* Typhimurium TA98, TA100, TA102, TA1535, TA1537, and *E. coli* (WP2uvrA) with and without metabolic activation. In vitro chromosome aberration test in Chinese Hamster Lung (CHL) was conducted at concentrations of 0, 1250, 2500, and 5000 g/ml.

Results: Chemical analysis showed that the major components of methanol extracts of *Cirsium japonicum* were flavones such as apigenin and luteolin. No significant adverse effect on body weight change, mortality, clinical signs, and gross findings was observed for 15 days after administration of a single dose. In a bacterial reverse mutation assay, *Cirsium japonicum* extracts ranging from 156 to 5,000 g/plate did not induce base-pair substitution or frame shift. Any significant increases in structural aberration as well as reduction of cell cycle progression were not observed in chromosomes.

Significance: Results from our study illustrated that methanol extracts of *Cirsium japonicum* containing flavones were not shown to induce oral acute or mutagenic toxicity.

P1-55 Effect of Acids on Optimum pH of Benzene Formation

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Introduction: Benzene is a carcinogen, and one way it is formed is by the reaction of benzoate salts and ascorbic acid. The upper limit of benzene allowed in drinking water is 5 ppb. In 2006, FDA examined more than 100 soft drinks containing benzoate salts and ascorbic, and showed that the benzene level in some of these samples was higher than the limit. The various benzene levels were explained on the basis of its being affected by storage conditions, temperature and sunlight.

Purpose: To better understand the conditions, including type of acid and pH range, for maximum and minimum benzene formation by benzoate salts and ascorbic acid in beverages. Results could be used to advise the beverage industries on formulation and storage.

Methods: Static headspace and gas chromatography-mass spectrometry were employed to determine the benzene concentration in the samples.

Results: One of the results obtained from the study shows that there is a narrow pH range producing maximum benzene yield from benzoate salts and ascorbic. At the optimum pH, the formation of benzene is up to 4 times higher than that at the neighbor points, which has not been reported in the literature. Organic and inorganic acids were used to adjust the pH for the investigation. Results indicate that each type of acid shows a different optimum pH of benzene formation. Therefore, there is a narrow pH range for maximum benzene formation, and there is also a pH range for minimum benzene formation. These results could be used to explain excessive benzene generated during storage, which could be the solution's pH shifts to the optimum pH and could be used in the formulation of beverage to avoid the optimum pH so that the benzene formed in the beverage is below the allowed permit limit.

Significance: Results indicate that each type of acid shows a different optimum pH of benzene formation. These results could be used to explain excessive benzene generated during the storage, which could be the solution's pH shifts to the optimum pH. And it could be used in the formulation of beverage to avoid the optimum pH of benzene formation.

P1-56 Dietary Exposure to Chloropropanols of Secondary School Students in Hong Kong

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Introduction: Chloropropanols, including 3-monochloropropan-1,2-diol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP), were originally identified as contaminants of the savory ingredient, acid-hydrolyzed vegetable protein. They can also form in food as the result of heat processing, as well as other processing or storage conditions.

Purpose: The purpose of this study was to examine the levels of 3-MCPD and 1,3-DCP in a wide range of food items, to estimate the dietary exposure to the above two chloropropanols of secondary school

students in Hong Kong and to assess the associated health risks based on the available data.

Methods: Dietary exposure to chloropropanols was estimated using the local food consumption data obtained from secondary school students in 2000 and the concentrations of 3-MCPD and 1,3-DCP in food samples taken from the local market. Laboratory analysis for 3-MCPD and 1,3-DCP was conducted by the Food Research Laboratory of the Center for Food Safety.

Results: The dietary exposure to 3-MCPD for an average secondary school student was estimated to be 0.063–0.150 micrograms per kilogram of body weight per day while that for the high consumer was 0.152–0.300 micrograms per kilogram of body weight per day. Both estimates fell below the provisional maximum tolerable daily intake of 2 micrograms per kilogram of body weight established by JECFA and amounted to less than 20% of this safety reference value. The dietary exposure to 1,3-DCP for an average secondary school student was estimated to be 0.003–0.019 micrograms per kilogram of body weight per day while that for the high consumer was 0.009–0.040 micrograms per kilogram of body weight per day. The resulting margin of exposures were of low concern for human health.

Significance: It could be concluded that both the average and high consumers among secondary school students were unlikely to experience major toxicological effects of 3-MCPD and 1,3-DCP.

P1-57 Mercury Levels in Female Students of the University of Japan – Relationship with Contents of Meals

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Introduction: Mercury is known to have detrimental effects on the nervous system; therefore, the effect of mercury on fetuses is a cause for great concern. Since approximately 90% of the dietary sources of mercury are fish products, it is necessary to be careful about their intake.

Purpose: In this study, we investigated the mercury levels in young women who had never undergone pregnancy and in their meals, and aimed at clarifying the relationship between mercury level in humans and in their meals.

Methods: We recruited 113 female university students (age 19–21 years) in June 2007 and carried out a questionnaire survey on the contents of their meals. Simultaneously, we collected hair samples and measured the mercury concentration in the samples by the cold-vapor atomic absorption method. Additionally, we investigated the mercury levels in mackerel, a fish reported to be present at very high levels in the diet, and examined the efficiency of mercury removal by cooking of fish according to various recipes.

Results: The mercury concentration in the hair of the subjects was 0.20–3.92 ppm, and the mean concentration was 1.37 ppm. This value was considerably lower than 50 ppm, the concentration considered to be the upper limit of the tolerable levels of mercury. In addition, the mercury levels tended to fall slightly from summer to autumn. The results of the questionnaire on the contents of their meals and the analysis of the hair samples indicated that the students who preferred fish products had higher mercury contents in their hair. We found that among the different cooking methods, pickling in vinegar could reduce the total mercury concentration by 40%.

Significance: The development of a cooking method that is highly effective in removing mercury in fish meat is important to protect a fetus from mercury toxicity.

P1-58 Fish Consumption by Mothers of Infants and by Women of Childbearing Age

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Introduction: Fish consumption by women throughout childbearing years has been suggested to confer several benefits to fetus and baby. However, certain fish are known to contain high levels of methyl mercury, which is a neurotoxin. FDA recommends that pregnant women and women of childbearing age consume up to 12 ounces of seafood per week and avoid consuming certain fish high in methyl mercury.

Purpose: The study evaluates levels of fish consumption among pregnant women, post-partum women and a comparison group of non-pregnant women of childbearing age.

Methods: The Infant Feeding Practices Study II (IFPS II) is a longitudinal, mail study of pregnant women and mothers of healthy, singleton infants sampled from a nationally distributed, but not representative, consumer panel. The diets of women in the study, as well as of a group of non-pregnant women, were assessed using Diet History Questionnaires (DHQ). Women were asked about the types of seafood, if any, they had eaten during the past month, as well as portion sizes and frequency.

Results: Most women in each group had consumed some seafood during the past month (79.3% of pregnant, 80.0% of post-partum, and 83.1% of the comparison group). Weekly median intakes of total seafood for the groups were 1.89, 2.17, and 2.97 ounces per week, respectively. A small proportion of each group (less than 25%) consumed more than 5 ounces of seafood per week. A majority consumed canned tuna fish, a good source of long-chain omega-3 fatty acids (54.0% of pregnant, 53.3% of post-partum, and 57.7% of comparison group), with median weekly intakes of 1.33 ounces for the pregnant and post-partum groups and 1.26 ounces for the comparison group. The percentage of women who had consumed fish high in methyl mercury was low in all three groups (1.7%–4.7%).

Significance: The data suggest that a low percentage of women in each of the groups consume more than the FDA recommended limit of 12 ounces of seafood per week. A very small percentage of women consume fish that are relatively high in methyl mercury.

P1-59 Responses of *Listeria monocytogenes* to Disinfection Stress Monitored by Measurements of Intracellular pH and Viable Counts

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Introduction: Subtypes of the human pathogen *Listeria monocytogenes* can persist in food processing plants for many years, which could be due to increased disinfectant tolerance.

Poster

Purpose: The purpose of this study was to determine the effect of disinfection stress at the single cell level for a persistent *L. monocytogenes* and possible occurrence of persister cells. The influence of sodium chloride on disinfection tolerance was also assessed.

Methods: Planktonic cells of *L. monocytogenes* were grown with and without NaCl and mixed with three concentrations (0.0015%, 0.0031%, 0.0062%) of an acidic disinfectant, Incimaxx, using water as control. The response of *L. monocytogenes* was studied as to both with viability and intracellular pH (pHi). Bacterial numbers were quantified with plate counting. pHi was measured following the same single cells over time with Fluorescence Ratio Imaging Microscopy.

Results: pHi values decreased in *L. monocytogenes* during exposure to all three concentrations of Incimaxx. The response of the bacterial population was homogenous since the pHi decreased to the same values ± 0.5. Hence, persister cells were not detected. Growth with NaCl protected the bacteria against disinfection since pHi was higher (6–6.5) when the bacterial population grown with NaCl was treated for 20 min with Incimaxx (0.0015%) as compared to cells grown without NaCl (pHi = 5–5.5) ($P < 0.05$). However, the bacteria survived the low pHi since the viable count was not different from the control treatment with water ($P > 0.05$). The protective effect of NaCl was reflected by viable counts at a higher concentration of Incimaxx (0.0031%) where the salt-grown population survived better (10^8 CFU/ml) than the population grown without NaCl (10^5–10^6 CFU/ml) ($P < 0.05$).

Significance: A population of a persistent *L. monocytogenes* is homogenous with respect to sensitivity to an acidic disinfectant studied at the single cell level; hence persister cells do not appear. However, exposure of the bacteria to a common food component increases their disinfection tolerance.

P1-60 Characterization of Yeast Heterogeneity and Succession during the Spontaneous Fermentation of Natural Black Olives

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Introduction: Natural black olive fermentation is one of the most important elaboration processes based on a complex microbial flora made up principally of yeasts, along with Gram negative bacteria and lactic acid bacteria to a lesser extent. Despite the fact that the contribution of the yeast community has been investigated as to both negative (softening, gas-pocket formation) and positive aspects (organoleptic profile), the characterization and/or identification of this population has been overlooked. The information on this issue is mainly based on conventional taxonomic studies with classical methodologies. Thus the biodiversity of this dynamic flora is not fully exploited.

Purpose: The purpose of this study was the identification and characterization of the indigenous yeast population dynamics during the spontaneous fermentation of natural black olives in brine.

Methods: Natural black olives were subjected to traditional anaerobic fermentation. Brine samples were routinely analyzed microbiologically (yeasts, enterobacteria, lactic acid bacteria) and physicochemically (pH, acidity) for a period of 30 days. Yeast diversity was evaluated by applying Restriction Fragment Length Polymorphism (RFLP) and sequence analysis of the 5.8S ITS and the D1/D2 ribosomal DNA regions of isolates. Yeasts subjected to molecular analysis were isolated from the brines at the beginning (day 0), middle (day 13) and final (day 28) stages of fermentation.

Results: Analysis revealed a relatively broad range of biodiversity composed of 8 genera and 11 species. In the beginning of the process, yeasts were enumerated at 3.5 $\log_{10}$ CFU ml^{-1} with *Metschnikowia pulcherrima* being predominant (50% of isolates) followed by *Debaryomyces hansenii* (11%), *Auerobasidium pullulans* (11%), *Pichia kluyveri* (3%), *Rhodotorula mucilaginosa* (7%) and *Rhodosporidium diobovatum* (18%). A structural alteration of the yeast community was observed as the fermentation proceeded. Yeast population increased gradually to 4.7 and 4.1 $\log_{10}$ CFU ml^{-1} in the middle and last stages, respectively. *P. membranifaciens* evolved as the central microorganism in olive fermentation (40–50% of isolates), prevailing at both stages of the process. *P. anomala* and *Candida boidinii* also accounted for a significant proportion of the total yeast population at these fermentation stages.

Significance: The present results will help to elucidate the implication of different yeast species in natural black olive fermentation and their potential contribution to the final characteristics of the fermented product.

P1-61 Impact of Teichoic Acids D-Alanylation in *Lactococcus lactis* on Its Surface Physicochemical Properties, Adhesion Behavior and Nisin and Lysozyme Sensitivity

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Introduction: The Gram-positive cell wall is formed by a thick peptidoglycan layer, decorated by proteins, polysaccharides and mainly by polymers of alternating phosphate and alditol groups called teichoic acids (TAs). TAs are either covalently linked to the peptidoglycan (wall teichoic acids, WTAs), or to membrane glycolipids (lipoteichoic acids, LTAs). They confer high densities of negative charges (due to the presence of phosphate groups) in the cell envelope. TAs are usually esterified with D-alanine, which results in a decrease of the charge of the TAs, since the positively charged amino groups of D-alanyl esters partially counteract the negative charges of the backbone phosphate groups.

Purpose: The aim of this study was to examine the impact of D-alanylation of TAs in *Lactococcus lactis* on the physicochemical properties of the bacterial surface and on bacterial adhesion on solid surfaces.

Methods: In *L. lactis*, the enzymes that catalyze teichoic acid D-alanylation are encoded by the genes of the dlt operon. We overexpressed the dlt operon of *L. lactis* MG1363 by cloning it in a multicopy plasmid, either with its own native promoter, or downstream of a nisin inducible promoter.

Results: Both mutant strains were found to present increased D-alanylation of their TAs, which was accompanied by a significant increase of their resistance towards the cationic antimicrobials, nisin and lysozyme. The opposite pattern was observed with a dltD negative mutant strain. Interestingly, no differences at bacterial cell surface hydrophobicity and charge were observed between mutant and wild type strains. Adhesion to two model surfaces (polystyrene and glass) was not modified either.

Significance: Our data suggest that, regarding *L. lactis*, the modification of the antimicrobial resistance results from variations of TA borne negative charges that are embedded inside the cell wall, rather than to the modification of the global charge of the bacterial surface.

P1-62 Assessment of Microbiological Quality and Food Safety Management Performance for School Food Service in South Korea

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Introduction: The school foodservice indicated a 100% participation rate in elementary, 99.3% in secondary, and 95.1% in special schools. The implementation of the school sanitation program based on the Hazard Analysis Critical Control Point (HACCP) has been followed to prevent foodborne disease since 2000 in South Korea.

Purpose: The purpose of the study was to evaluate the microbiological quality, diagnose the sanitation program of current school foodservice operations and propose improvements in sanitation management.

Methods: Microbiological quality was assessed using 3M Petrifilm™ to measure total plate count, coliforms, *E. coli*, *S. aureus*, and *Enterobacteriaceae* for samples. The microbiological hazard analysis is focused on gloves, knives, dish cloths, utensils for serving, raw food and pre-prepared food ingredients. The school foodservice sanitation inspection was carried out at four self operated elementary and six secondary schools located in Seoul and Gyeonggi province.

Results: The TPC counts from pre-prepared bracken, bellflower root and pickled cucumber were relatively high raging from 4.4 to 9.08 log CFU/g. The microbial result of *Enterobacteriaceae* in bellflower root (7.26 log CFU/g) and TPC in prickled cucumber (TPC: 4.24 log CFU/g) after sanitation exceeded standards (*Enterobacteriaceae*: 2 log CFU/g, TPC: 6 log CFU/g, according to the sanitation program. The results of analyzing and evaluating each of the items through the sanitation inspection showed the average of the ten schools to be 82.7 points; the average score (% of compliance) of each field was 8.7/9 (96.7%) for facilities management, 4.5/6 (75%) for personal hygiene, 5.4/6 (90%) for ingredient control, 20.4/24 (85%) for production environment, 5.4/6 (90%) for serving, 4.8/6 (80%) for washing and sanitation, and 7.8/9 (86.7%) for safety management.

Significance: These results suggest that proper selection of providers and good practice of sanitation processes are necessary to control the microbial hazard of pre-prepared produces. Also, the employees should take effective training and education to understand and maintain personal hygiene.

P1-63 Efficacy of Electrolyzed Oxidizing Water against *Listeria monocytogenes* and *Morganella morganii* Biofilms

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Introduction: Listeria monocytogenes and *Morganella morganii* have been implicated in listeriosis outbreaks and histamine fish poisoning, respectively, from consumption of smoked fish. Possible sources of contamination include processing equipment, food handlers, and smoke houses. Treatment of food preparation surfaces and of whole fish during handling with agents such as electrolyzed oxidizing water (EOW) could reduce biofilm formation on seafood products and in seafood processing plants.

Purpose: We examined the efficacy of EOW against *L. monocytogenes* and *M. morganii* biofilms, using the MBECTM Assay System (Innovotech Inc.).

Methods: The MBEC™Assay System was used to assess the activity of EOW against 24-h biofilms of 114 *Listeria* isolates (primarily *L. monocytogenes*), 5 strains of *M. morganii,* and 10 other bacterial species. EOW was produced using the ElectroCide System (Electrolyzer Corp.). Biofilms were exposed to EOW for 0 (control), 5, 15, and 30 min. A duplicate set of biofilms was also tested after pre-treatment with the alkaline water produced by the electrolysis process, as suggested by the manufacturer.

Results: Approximately 1×10^7 CFU/ml of *L. monocytogenes* or *M. morganii* cells were recovered from untreated (control) biofilms. Results (recovery vs. no recovery) obtained following treatment for 5 or 15 min were strain-dependent. No *L. monocytogenes* or *M. morganii* cells were recovered after 30 min of EOW treatment of biofilms, with or without pre-treatment.

Significance: These results suggest that exposure to EOW for at least 30 min can remove existing biofilms. Continuous or intermittent spraying of food processing equipment (e.g., conveyor belts, slicers) could prevent further biofilm formation.

P1-64 Assessment of Microbial Contamination Levels of Street-Vended Foods in Incheon and Daegu, Korea

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Introduction: Street-vended foods are defined as foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation. Street-vended foods are often prepared in unhygienic conditions and consequently contaminated with food poisoning pathogens. Therefore, there is a need to monitor the microbial safety of street-vended foods to ensure the protection of the consumer.

Purpose: The objective of this study is to assess the level of microbial contamination of street-vended foods in Incheon and Daegu, Korea.

Methods: Samples of street-vended foods (n=326) were collected from Incheon, Deagu, Bucheon and Gumi. The types of food samples collected were fruit juice, fried foods, sandwich, toast, hamburger, Kimbab (rice rolled in dried laver), Ggochi (spicy chicken and vegetable barbecue), Dduckhokggi (broiled dish of sliced rice cake, vegetable, seasoning, etc.), Odeng (boiled fish surimi) and Sundea (a sausage made of bean curd and green-bean sprouts stuffed in pig intestine). The foods were tested for *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Samonella* spp., *Listeria monocytogenes*, aerobic mesophilic bacteria and total coliforms.

Results: Contamination levels of *E. coli*, *S. aureus*, aerobic mesophilic bacteria and total coliforms in various street-vended foods were 0–3.39, 0–4.15, 0–7.43, 0–6.86 $\log_{10}$ CFU/g, respectively. *V. parahaemolyticus*, *Salmonella* spp., and *L. monocytogenes* were not detected. Mean values of $\log_{10}$ CFU/g by foods were aerobic mesophilic bacteria: Kimbab (6.03) > Hamburger (5.14) > Sandwich (4.88) > Fruit juice (4.82) > Sundea (4.39) > Ggochi (4.30) > Fried (4.13) > Toast (3.71) > Dduckbokgg i(3.67) > Odeng (3.00), Total coliforms: Hamburger (4.14) > Kimbab (3.75) > Sandwich (3.62) > Dduckbokggi (3.09) > Fruit juice (2.91) > Sundea (2.60) > Ggochi (2.45) > Fried (2.35) > Toast (2.24), *E. coli* : Sundea (2.42) > Fried (2.30) > Fruit juice (1.93), *S. aureus* : Fried (4.15) > Kimbab (3.35). To characterize *E. coli* (n = 10) and *S. aureus* (n = 30) isolates, we evaluated the presence of major virulence-associated genes and ability for toxin production using PCR and RPLA. Among enterotoxin-encoding genes, *Staphylococcus* enterotoxin type A (SEA) was detected in 17 isolates and both SEA and *Staphylococcus* enterotoxin type B (SEB) were detected in 2 isolates.

Significance: This study can be utilized to provide information on the hygienic condition of street-vended foods and to improve the safety of street-vended foods in Korea.

P1-65 DSC Evaluation of the Effectiveness of Prerequisite Programs and HACCP in Small Size Food Service Establishments in Birmingham, United Kingdom

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Introduction: HACCP is a straightforward system that ensures food safety by reducing the incidents of food being contaminated by potential hazards. Before properly implementing a HACCP system, companies are required to have the prerequisite programs (PRP) in place, which ensures concentration on the most significant food hazards. As a result of European legislation, since January 2006, all food operators including small and medium enterprises (SMEs) are required to have a system based on HACCP principles in place.

Purpose: To assess the improvements in hygiene, by applying just PRP, or combined PRP & HACCP to SMEs, and determine whether the application PRP alone will be sufficient to give a measurable and significant improvement in hygiene for this type of businesses.

Methods: A longitudinal study was carried out in SMEs using 5 one-to-one coaching visits to help the implementation of the PRP and HACCP. PRPs were applied using Safer Food Better Business (SFBB). This is generic guidance developed by the Food Standards Agency in England for small catering businesses. Further assistance was given to develop a HACCP plan specific to their operation. The evaluation consisted of audits, environmental samples, knowledge assessments and qualitative surveys as indicators of hygiene.

Results: It was found that a statistically significant improvement ($P < 0.05$) in hygiene was achieved using the PRP (SFBB). No further significant additional improvement could be measured after the implementation of a HACCP plan into the SME food service establishments.

Significance: The use of PRPs would appear to achieve the biggest improvement in food safety control in SMEs, implying that the additional costs of HACCP implementation may not be warranted.

P1-66 DSC Monitoring of Hand Sanitation of Employees in Public Restaurants, and Evaluation of Hand Washing Methods to Reduce Bacterial Populations on Their Hands

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Introduction: Hand washing is one of the critical factors to improve food safety in public restaurants. Therefore, it is necessary to evaluate levels of bacterial populations on hands and plastic gloves of restaurant employees.

Purpose: Objectives of this study were to monitor levels of aerobic plate counts (APCs) and coliform bacterial populations on kitchen workers' hands and plastic gloves, and to evaluate washing method of hands and plastic gloves.

Methods: To monitor sanitation levels of employees' hands, samples of employees' hands and plastic gloves were collected using the Glove juice method in five public restaurants in Jinju, South Korea. Microbial samples were collected every 30 min for 180 min of working hour to estimate increases in bacterial populations on hands and gloves. Moreover, 20 s washing effects of water, soap (bar type), and soap and 70% alcohol were examined on plastic gloves used for vegetables, meat and fish. APCs and coliform bacterial populations in the collected samples were enumerated on plate count agar and desoxycholate lactose agar, respectively.

Results: APCs and coliform bacterial populations on employees' hands were 5.3–5.8 log CFU/hand and 3.2–3.7 log CFU/hand, respectively, while 4.4–5.2 log CFU/hand of APCs and 3.1–4.1 log CFU/hand of coliform bacteria were recovered from employees' plastic gloves. The bacterial populations on hands of kitchen workers increased significantly ($P < 0.05$) after 1.5 h of working h. Reductions (0.9–1.6 log CFU/hand) were not significant ($P > 0.5$) among washing methods for coliform bacteria, while hand washing methods using soap (1.0–2.8 log CFU/hand) reduced APCs more ($P < 0.05$) than water (0.8–1.2 log CFU/hand), but no difference ($P > 0.5$) was found between soap, and the combination of soap plus 70% alcohol.

Significance: These results suggest that hand sanitation in public restaurants should be improved, and application of soap should be effective to improve kitchen workers' hand sanitation. Results from this study should also be useful to develop hand washing standards.

P1-67 Analysis of School Foodservice Dietitians' Perception of Barriers to HACCP Implementation and Food Sanitation/Safety Management Performance in South Korea

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Introduction: Hazard Analysis Critical Control Point (HACCP) has been implemented since 2000 to reduce the prevalence of foodborne diseases in South Korea's school foodservices. Today, school foodservice safety programs based on HACCP are applied to 99.6% of schools (10,986 schools) in South Korea. To successfully implement HACCP and maintain the safety of school

foodservices, barriers to the HACCP system and food safety programs must be determined.

Purpose: The purpose of this study was to identify barriers to HACCP implementation and to investigate the relationship between dietitians' perceptions of barriers to HACCP implementation and food sanitation/safety management performance within school foodservices.

Methods: 797 school dietitians (9.3% of total school dietitians) in South Korea were surveyed with the cooperation of 16 District Offices of Education, which supervise school foodservices throughout the nation. Respondents were asked to assess obstacles to HACCP implementation, and food sanitation management performance, and to provide demographic information. For statistical analysis, SPSS Windows (version 12.0) was used to conduct descriptive statistics, and to perform *t*-tests, and Pearson correlations.

Results: Dietitians perceived 'lack of budget' and 'limited availability of facilities/equipment' as the biggest obstacles to implementing HACCP. The dietitians who worked at self-operated foodservices perceived four out of seven barriers to HACCP implementation as higher than these were perceived by contract-managed dietitians, while contract-managed dietitians perceived only one category as higher than dietitians who work at self-operated foodservices ($P < 0.05$). Dietitian perceptions of barriers to HACCP implementation were correlated to the food sanitation/safety management performance; when dietitians perceived facilities/equipment-related barriers as greater, performance of 'facility management' and 'safety management' were significantly lower ($P < 0.001$).

Significance: These data suggest that more investment in facilities/equipment is needed for food safety improvement and successful HACCP implementation in school foodservices. Improvement of facilities/equipment will lead to improved facility and safety management.

P1-68 Evaluation of Sanitation Practices in South Korean School Food Service Facilities

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Introduction: Foodborne illness outbreaks associated with foods prepared in school food facilities in South Korea have increased in recent years. Development of appropriate intervention strategies require an assessment of potential causes of these outbreaks such as general sanitary conditions.

Purpose: This study evaluated sanitary conditions in school foodservice facilities.

Methods: Microbial samples were collected in summer and winter months from five school foodservice facilities in Jinju, South Korea (two replicates/foodservice/season). Tap and drinking water samples were collected from each facility, and sterile swabs were used to collect surface samples of cooking utensils (cutting boards, knives, tongs, bowls, baskets), tableware (food serving trays with indentations, spoons and chopsticks, and stainless steel cups), surroundings (serving counters, disinfection cabinets, refrigerators, ventilation fans, and sinks), linen (fabric towels), and employee aprons. Samples of employees' hands and plastic gloves were also collected using a Glove juice method. Aerobic plate counts (APC, plate count agar [PCA]), and coliform counts (desoxycholate lactose agar) were determined, and the presence of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* were evaluated after sample enrichment. Suspect isolated were confirmed by biochemical tests. APC, fungi (Rose Bengal Agar) and *S. aureus* (Baird-Parker Agar) counts were determined for air by leaving plates open in the kitchen for 15 min.

Results: APCs and coliform counts in water were 0–5.1 and ≤ 2.0 log CFU/ml, respectively. Microbial populations were highly variable but in general when colonies were detected, APC and coliform counts were 1.4 to 5.7 and 1.0–4.8 log CFU/40 or 100 cm^2, respectively. Microbial populations were detected for hands, cooking utensils, tableware, several surrounding surfaces, and linen. Seasonal effect ($P < 0.05$) was observed only in coliform counts on cutting boards and baskets. *Salmonella* and *Listeria monocytogenes* were not isolated from any of the samples. *S. aureus* was detected in 10–30% of samples including water, cooking utensil, tableware, employee hands or gloves and surroundings. *E. coli* (10–20%) was isolated from most cooking utensils. Colonies were not detected in plates exposed to air.

Significance: These results highlight that sanitation practice in school foodservice facilities should be improved and that microbial monitoring may be a useful assessment tool.

P1-69 A Novel Kitchen Disinfectant Effective against Norovirus

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Introduction: Only a few disinfectants are known to be effective against norovirus. Free chlorine is recommended and widely used for disinfection; however, it loses its virucidal activity in the presence of a small amount of organic compounds.

Purpose: To develop a kitchen disinfectant effective against norovirus and other foodborne pathogens, which is safe to be used in food-service industries.

Methods: Feline calicivirus (FCV), a norovirus surrogate, was cultivated in feline kidney cells. The viruses were prepared by differential centrifugation and suspended in a saline solution. The suspension was mixed with various disinfectants at a ratio of 1:9 and reacted for 30 s. The mixtures were then serially diluted and inoculated onto cells in multiplates. The cells were fixed and stained to determine the infectivity after cultivation for four days.

Results: Phenolic compounds were not effective enough against FCV, though several studies have proved their effectiveness. Ethanol reduced the infectivity of the virus by only 1 to 2 logs when used alone. However, its activity was increased when the pH was raised to higher than 8 by addition of weakly alkaline inorganic salts and adjusted with organic acids and their alkali metal salts. Further addition of nonionic surfactants increased the activity even more. A mixture consisting of 58 vol% ethanol, nonionic surfactants, inorganic salts, organic acids and their alkali metal salts inactivated FCV and *E. coli* by more than 4 and 5 logs, respectively. The activity of the disinfectant mostly remains despite addition of bovine serum up to 5%.

Significance: The disinfectant is composed only of food additives approved in Japan and thus expected to be useful in food industries.

P1-70 Efficacy of Aerosolized Sanitizers on the Inhibition of Bacterial Biofilms

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Introduction: Various bacteria, including food spoilage bacteria and pathogens, could form biofilms on various food processing surfaces, potentially leading to food contamination.

Purpose: This study evaluated the efficacy of aerosolized commercial sanitizers to inhibit various kinds of bacterial biofilms, using a polyvinyl chloride (PVC) microtiter plate assay.

Methods: Various concentrations (10^0–10^9 CFU/ml) of 8 different bacteria (*Bacillus cereus, Escherichia coli, E. coli* O157:H7, *Klebsiella pneumonia, Listeria monocytogenes, Pesudomonas aeruginosa, Stapylococcus aureus*, and *Salmonella* Typhimurium) in TSB were placed in PVC microtiter plate wells and incubated at 22°C for 24 h to form biofilms. Microtiter plate wells were then washed and dried followed by treatment with aerosolized hydrogen peroxide-based and quaternary ammonium-based sanitizer in a model cabinet for 30 or 60 min at room temperature. Biofilm concentrations were indirectly assessed by staining with 1% crystal violet.

Results: Treatment with aerosolized hydrogen peroxide-based sanitizer for 30 and 60 min effectively removed all bacterial biofilms except for *P. aeruginosa*. Treatment with aerosolized quaternary ammonium-based sanitizer for 30 min was effective at eliminating bacterial biofilm by *B. cereus*, *L. monocytogenes*, *S. aureus,* and treatment for 60 min was effective for removing *K. pneumoniae* biofilm.

Significance: In summary, aerosolized sanitizers have great potential for use in food environments against bacterial biofilms. However, more detailed studies in food models are required prior to commercial application.

P2-01 Genetic Diversity of *Alicyclobacillus acidoterrestris* and the Correlation with Their Spoilage Ability

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Introduction: Alicyclobacillus are Gram-positive, moderately thermophilic, acidophilic, endospore-forming bacteria that sometimes cause spoilage of beverages, forming odorous compounds, including guaiacols. *Alicyclobacillus acidoterrestris*, the most frequent odor producing species, is the main target species within the genus which is of quality concern in the beverage industry. However, the spoilage pattern varies depending on factors such as strain, beverage type, and storage conditions.

Purpose: The purpose of this study was to investigate intraspecies genotypes of *A. acidoterrestris*, and elucidate the correlation of phylogenetic relatedness and their spoilage ability within/among the intraspecies genotype groups.

Methods: Phylogenetic analysis based on 16S rRNA gene sequence (approximately 1,500 bp) was performed on 197 strains of *A. acidoterrestris* isolated from beverages and their raw materials. The ribopatterns of the 197 isolates was also analyzed and compared to the result of the 16S rRNA gene-based dendrogram.

Results: According to the phylogenetic analysis, the 197 isolates were partitioned into four phylogenetically distinct groups (A-D). While Group A (73 strains) was composed of strains with high sequence similarities, Group D (120 strains) consisted of rather different strains. Group B (2 strains) and Group C (2 strains) were composed of only two strains each and did not show a close relationship with any other groups. According to the ribotyping analysis, the ribopatterns of Group A were also highly conserved, but Group D showed diversity in their ribopattern. Comparison of the ribopatterns with 16S rRNA gene-based clusters showed that most ribopatterns were in good agreement with 16S rRNA gene-based clustering, but some ribopatterns disagreed with 16S rRNA gene-based phylogenetic relatedness.

Significance: According to these results, *A. acidoterrestris* isolates showed rich genetic diversity. This study provides fundamental data for investigation of variation in the spoilage ability of *A. acidoterrestris*.

P2-02 Survey of Yeast and Mold Found in Food Bought at Retail

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Introduction: Fungal contamination is a major cause of economic loss to the food industry. Preventing spoilage often requires identification of the offending organism so that adequate barriers can be implemented. Fungi (yeast and molds) are an enormously diverse group comprising on the order of one million distinct species, the vast majority of which are yet to be identified. Conversely, many of the more common species have been given multiple names, in part because of the difficulties inherent in morphological identification.

Purpose: To examine the diversity of fungal species present in retail food.

Methods: Over 400 food items from a variety of product types were purchased from stores in Delaware and Pennsylvania. Items were screened for high levels (> 100 CFU/g) of yeast or mold by homogenization in phosphate buffered saline and plating the equivalent of 0.01 gram of product on non-acidified PDA plates with Chloramphenicol and tetracycline. When multiple colony morphologies were observed, the morphologically distinct colonies were isolated independently. A portion of the ribosomal RNA gene from each isolate was amplified and sequenced; the sequences were then compared to the NCBI database for identification.

Results: Over 70 species were isolated, including several apparently novel species, one of which likely represents a novel genus, based on genetic distance from previously reported species.

Significance: Sequence-based identification reveals a rich diversity of fungi in retail food items, with both common food-associated and completely novel fungal species identified.

P2-03 DSC Production of Shelf-Stable Ranch Dressing with Elevated pH Using Ultra-High Pressure

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Introduction: Ranch dressing is a complex food system that supports the growth of spoilage-inducing lactic acid bacteria and yeasts. Commercial production of shelf-stable dressing relies on acidification and preservatives to control microorganisms. However, consumers prefer premium quality dressings that are less sour, while industry desires extended shelf life. Thermal processing methods, while effective at inactivating microorganisms, cause emulsion instability and are therefore inappropriate for these products.

Purpose: This study determined the efficacy of ultra-high pressure (UHP) to inactivate spoilage-causing microorganisms and to extend the shelf life of premium quality ranch dressing. The impact of UHP on sensory, chemical, and physical attributes were evaluated.

Methods: Ranch dressing (pH 4.4) was inoculated with spoilage-causing *Pediococcus acidilactici*, *Lactobacillus brevis*, or *Torulaspora delbruekii*, treated with UHP (600 MPa; 3, 5, 10 min) and stored at 27°C for 25 days, and recovery of spoilage microorganisms was monitored. Suitable processing conditions deduced from the first experiment (600 MPa 5 min) were used to produce ranch dressing; the product was stored at 4°C, 27°C and 37°C with microbial, physical, chemical, and sensory analyses performed throughout the storage period (26 weeks) to determine acceptability following pressure treatment.

Results: Spoilage microorganisms varied significantly in their barotolerance; *P. acidilactici* was the most barotolerant and *Tp. delbrueckii* was the most sensitive. Shelf-life studies of uninoculated UHP-processed product determined the dressing to be microbiologically and physically stable. Organic acid profiles changed with extended storage and sensory analysis indicated product deterioration with extended storage at 27°C and 37°C, typical of traditionally produced dressings.

Significance: Ultra-high pressure processing is effective to produce premium quality ranch dressing without causing emulsion instability. *P. acidilactici* is barotolerant and would be an ideal target organism for process validation of ranch dressing.

P2-04 Investigation for Possible Sources of Contamination of Spoilage Microflora Associated with "Blown-Pack" Spoilage of Ground Beef Chubs

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Introduction: "Blown-pack" spoilage of ground beef chubs is characterized by swelling of chub-packaged ground beef products and results in substantial economic losses annually in the beef industry.

Purpose: The objective of this study was to identify harborages and points of increased risk for product contamination with potential gas-producing spoilage organisms in a meat processing environment.

Methods: Environmental swabs (N = 643) were collected from the meat processing environment (carcasses, floor, drains, conveyor belts, tables, and employee equipment) by using sterile sponges moistened with neutralizing buffer. Product samples (N = 280) (meat trim and purge) and carcass swabs (N = 280) were also collected from different areas (carcass transfer area, fabrication room, and grinding room) of the processing plant, using sterile stomacher bags. Environmental and product samples were transported to the laboratory in coolers, stored at 4°C, and analyzed within 24 h. Samples were enriched using gas assay media containing Durham tubes and incubated at 7°C until visual signs of gas production were observed. Gas-positive samples were streaked onto seletive agars for isolation. The isolated organisms were identified by phenotypic characterization and 16s rRNA sequencing.

Results: In the carcass transfer area, 50% of touching carcasses sampled yielded organisms with gas production while 0% of non-touching carcasses displayed gas production. Swab samples (25%) of hotbox double-doors demonstrated gas production. In the fabrication area, samples from trim catch points, return belt rollers, hollow frame work, and cutting boards showed gas production. Organisms from product contact surface samples (25%) and environmental samples (25%) collected from the grinding room showed gas production. *Enterobacteriaceae* (56%), lactic acid bacteria (25%), and *Aeromonas* spp. (19%) were the predominant gas-producing spoilage microflora identified on both environmental and product samples.

Significance: The current validation study allows meat processors to identify possible harborages and points of increased risk for product contamination with potential gas-producing spoilage organisms.

P2-05 Microbial Quality of Beverages Sold by Fast Food Restaurants and Convenience Stores in Griffin, Georgia and Surrounding Areas

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Introduction: Dispensing machines in fast food restaurants and gas station convenience stores are popular places for Americans to purchase soft drinks.

Purpose: This study evaluated the microbial quality of 17 different types of beverages sold by the dispensing machines in 10 fast-food restaurants or gas-station convenience stores in Griffin, GA and surrounding areas.

Methods: Forty-three beverages were purchased in 2006 and in 2007, and 100 ml of each beverage was filtered through sterile, hydrophobic grid membranes. The remaining beverage was kept at room temperature for 4 h before sampling in order to mimic the possible holding time between purchase and consumption. The membranes were then sampled for total aerobes, *Enterobacteriaceae*, and lactic acid bacteria, as well as mold and yeast.

Results: The microbial counts from the beverages sampled in 2006 were numerically higher, except for the lactic acid bacterial counts, than those from the beverages sampled in 2007. Counts for the beverages sampled after the 4-h holding period were higher than for those sampled initially, except for the *Enterobacteriaceae* counts. The mold and yeast counts from the beverages sold by 4 and 3 of the venders were higher than 10^4 and 10^3 CFU/100 ml, respectively. The beverages from one of the fast food restaurants had the

highest *Enterobacteriaceae* and total plate counts, but their lactic acid bacterial counts were lower for beverages from one of the venders, and mold and yeast counts were lower than for beverages from 5 of the venders. A fruit-flavored beverage had 10^4 CFU/100 ml more bacterial cells than all 9 other types of beverages sampled, but its yeast and mold counts, also in the range of 10^4 CFU/100 ml, were the third highest among the tested beverages.

Significance: These results suggest that beverages sold by some of the venders in Griffin, GA and the surrounding areas in 2006 and 2007 may not meet the regulatory standards and consumer expectations.

P2-06 Withdrawn

P2-07 The Effect of Juice Temperature on *Clostridium botulinum* Type A Toxin Activity during the Hot Filling of Juice Bottles

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Introduction: The potential threat of terrorist attacks against the United States food supply using neurotoxin of *Clostridium botulinum* (BoNT) has resulted in the need for studying the effect of various food process operations on this toxin.

Purpose: The objective was to evaluate *C. botulinum* type A neurotoxin stability during the hot-fill juice bottling operation.

Methods: C. botulinum Type A Acid Mud Toxin (~10^6 MLD/ml) was deposited into juice bottles at the predetermined slowest heating spot. Twelve and twenty ounce bottles were filled with apple and orange-type juice at 85 and 80°C and held in upright and inverted positions. Toxin activity was tested as a function of holding time from 1 to 5 min by the Digoxigenin-ELISA assay (DIG-ELISA), confirming for toxin presence by mouse bioassay.

Results: For orange-type juice, pH 2.9, there was no recovered toxicity after 1 min of holding time for all bottle sizes, orientations, and temperatures from the DIG-ELISA and confirmatory mouse bioassays. This suggests there was at least a 0.5×10^6 MLD/ml reduction in activity. Inverted bottles of apple juice, pH 4.0, after 1 min of hold time at both temperatures had an apparent loss of toxicity of 0.25×10^5 MLD/ml. The upright orientation showed that the slowest heating zone was in the bottle's bottom rim for both sizes. For the upright orientation, the toxicity loss was lower than that of the inverted orientation. It appears that up to 2 min of hold time is required to deliver the same loss of toxicity as the inverted orientation causes.

Significance: Hot filling of juices at or above 80°C should render the product safe if up to 10^4 MLD of *Clostridium botulinum* type A toxin were added to bottles prior to filling.

P2-08 DSC Inactivation of *Listeria innocua* in Apple and Carrot Juices by High Pressure Homogenization and Nisin

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Introduction: High Pressure Homogenization (HPH) has been of growing interest as a nonthermal technology for microbial inactivation in fluid foods such as fruit juices. The proposed mechanisms behind its bactericidal effect include pressure, shear, turbulence, cavitation, and impact with solid surfaces, but are still not fully elucidated.

Purpose: The objectives of this study were to investigate the inactivation effect of HPH against *Listeria innocua* in apple and carrot juices and to examine the potential synergistic effect of the antimicrobial nisin associated with HPH.

Methods: L. innocua ATCC 51742 was inoculated into apple and carrot juice (10^7 CFU/ml) at ambient temperature containing 0 or 10 IU/ml nisin and then subjected to HPH from 350 to 0 MPa. At every 50 MPa of pressure step, a juice sample was collected and immediately cooled. Juice samples were serially plated in Tryptic Soy Agar supplemented with 0.6% yeast to enumerate survivors. Experiments were conducted in triplicate and analyzed as a randomized complete block design.

Results: L. innocua cells were able to survive HPH up to 200 MPa in both juices. The number of cells started to decrease considerably when pressures above 200 MPa were used. A > 5 log reduction in CFU/ml was observed when juices were subjected to 300–350 MPa. Inactivation was due to pressure and thermal effects. The addition of nisin did not have a synergistic lethal effect with HPH. In contrast to previous reports, data indicate that *L. innocua* is more resistant than other bacteria (e.g., *E. coli*) to HPH inactivation.

Significance: Results from this study suggest that the new technology, HPH, inactivates *Listeria innocua* by combined pressure and thermal effects. Validation studies using the pathogenic *Listeria monocytogenes* are still needed to fully demonstrate the potential of this technology to achieve the 5 log reduction required by current HACCP regulations for fruit juices.

P2-09 DSC Inactivation of *Escherichia coli* K-12 in Apple Juice and Apple Cider Using High Pressure Homogenization and Chitosan

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Introduction: Increased demand for natural, minimally processed products has led to the development of novel nonthermal preservation methods for inactivation of the microorganisms with minimal changes to the chemical and physiochemical qualities of fruit juices.

Purpose: The purpose of this study was to evaluate combined effects of high pressure homogenization and chitosan on the destruction of *Escherichia coli* K-12 in apple juice and apple cider.

Methods: Apple juice and apple cider were inoculated with *Escherichia coli* K-12 and processed using a high pressure homogenizer to study bacterial inactivation. Seven levels of pressure ranging from 50 to 350 MPa were used in the high pressure homogenizer. Two types of chitosan (regular and water soluble) with three levels of concentration (0, 0.01 and 0.1%) were investigated for synergistic effect with high pressure homogenization for the bacterial inactivation. *E. coli* K-12 inactivation was evaluated as a function of homogenizing pressure at different concentrations of chitosan in apple juice and cider.

Results: Homogenization at pressures of 100 to 200 MPa caused *E. coli* K-12 inactivation (1.3 to 4.15 log CFU/ml, respectively) with minimal increase in product temperature (< 64°C). Homogenization at

pressures >250 MPa resulted in significant increase in product temperature (>70°C) with concurrent microbial reductions (>7 log CFU/ml). Addition of chitosan at 0.1% concentration resulted in greater inactivation of *E. coli* K-12 in apple juice and apple cider homogenized with pressure up to 200 MPa. Interaction between the type of substrate (juice / cider), pressure and type of chitosan used was found to be significant during the study.

Significance: High pressure homogenization is a viable nonthermal alternative to thermal pasteurization for apple juice and apple cider.

P2-10 Development of a Rep-PCR DNA Strain-Typing Method for Members of the Genus *Alicyclobacillus*

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Introduction: Alicyclobacillus consists of a group of non-pathogenic, thermo-acidophilic, endospore-forming bacteria that cause sporadic spoilage of various shelf-stable juice products. These spore-forming soil microorganisms are primarily a cause of spoilage due to formation of guaiacol, a metabolic by-product. Spores can survive pasteurization treatment and tend to be resistant to common food preservatives. Most contamination problems occur several days after bottling liquid drinks, leading to off-flavor and potential product recalls.

Purpose: This study assesses the usefulness of an automated repetitive sequence-based PCR system, the DiversiLab System™, in the speciation and strain-typing of *Alicyclobacillus* spp. In addition, this study presents the clustering of guaiacol-producing isolates using the DiversiLab System. This System may provide a tool for fruit juice producers to monitor and control guaiacol-forming species.

Methods: Several *A. acidoterrestris*, *A. acidocaldarius*, *A. cycloheptanicus*, *A. herbarius*, *A. hesperidum* and *A. acidiphilus* isolates, including both guaiacol-producing and non-guaiacol producing isolates, were tested using the DiversiLab System. The isolates were cultured and genomic DNA was extracted from each culture, using the UltraClean™ Microbial DNA Isolation Kit. DNA was amplified using the DiversiLab Alicyclobacillus Kit for DNA Fingerprinting. The amplified product was analyzed using the DiversiLab System, which includes fragment separation using microfluidics chip technology and web-based data analysis software.

Results: Using the DiversiLab System, fingerprints were generated for every sample. The *Alicyclobacillus* kit discriminated among all species. In addition, there were clusters of guaiacol-producing strains.

Significance: The DiversiLab System is an effective method to distinguish between *Alicyclobacillus* species and shows potential for strain-level discrimination, suggesting the System can be used as a source-tracking tool to follow *Alicyclobacillus* strains during a contamination event.

P2-11 Rep-PCR Based Fingerprinting of *Saccharomyces* spp. Used in the Production of Fermented Beverages

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Introduction: The *Saccharomyces sensu* stricto species complex plays a crucial role in the production of fermented goods. Specifically, *S. cerevisiae* is used in the production of ales and wines, *S. pastorianus* in the production of lagers, and *S. bayanus* in the production of wines and ciders. The ability to distinguish these species from one another and to discriminate among strains within each species is important to brewers as well as to researchers. Hybridization among species, however, can make that endeavor difficult. Current methods of discrimination among and within these species include RAPDs, chromosomal karyotyping, RFLPs, and microsatellite analysis.

Purpose: The goal of this study was to assess the DiversiLab System™, an automated repetitive sequence-based PCR system, as a tool to discriminate among closely related strains and species of the genus *Saccharomyces*.

Methods: Thirty commercially available fermentation specific isolates of *S. cerevisiae*, *S. bayanus*, and *S. pastorianus* were cultured and genomic DNA was isolated using the UltraClean™ Microbial DNA Isolation Kit. DNA was amplified using the DiversiLab Saccharomyces Kit for DNA Fingerprinting. The amplified product was analyzed using the DiversiLab System, which includes the separation of amplicons with microfluidics chip technology and web-based data analysis software.

Results: Using the DiversiLab System, fingerprints were generated for every sample. The *Saccharomyces* Kit discriminated among species as well as among industrially relevant strains within these species.

Significance: These data suggest that the DiversiLab System is an effective tool for differentiating *Saccharomyces* species and providing strain-level discrimination among isolates used in the production of ales, lagers, ciders, and wines. The ability to rapidly and effectively strain-type these *Saccharomyces* spp. may be important for industrial quality control and protection of intellectual property of specific strains.

P2-12 Subclinical Mastitis in Dairy Ewes as a Source of Contamination for Bulk Milk and Cheese

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Introduction: Most ewes' milk cheeses (particularly those bearing a PDO status) are manufactured from unpasteurized milk, without deliberate addition of starter cultures; therefore, the microbiological quality of the cheesemaking milk is not only important to the evolution of cheese ripening, but also crucial to the microbiological safety of the final product.

Purpose: The major goal of this study was to ascertain the influence of subclinical mastitis in dairy ewes upon microbiological contamination of bulk tank milk and of cheese manufactured by use of traditional techniques.

Methods: Individual milk samples from each half-udder were collected from a total of 80 dairy ewes in a mixed-breed flock (including Assaf, Merino and Serra da Estrela breeds). Samples were collected at 2 different times within the milking season (winter and spring), for 2 consecutive years, from a group of 20 animals, and each was sampled twice throughout the lactation period (at 50 and 150 days). Bulk tank milk was collected at each of 8 sampling points, and cheese was manufactured from it following traditional practices;

bacteriological analyses were then performed on all samples.

Results: The lowest somatic cell counts were found in milk and 0 d-cheese, when low total microbial plate counts were also found, under regular ripening; these cheeses also presented lower counts of viable *Enterobacteriaceae*, yeasts and molds, and were devoid of visual and other sensory defects (unlike what happened with cheeses originated from bulk milk containing high somatic cell counts). The fraction of infected half-udders ranged from 20 to 78.9% and varied significantly ($P < 0.01$) between sampling seasons.

Conversely, the stage of lactation had no significant effect on the fraction of culture-positive milk samples.

Significance: The main risk factors in ewes' udder health are known to be environmental and management stressors; therefore, opportunistic and underlying infections should be regarded as a major contaminant source of ewes' milk cheese, and should consequently be prevented in order to achieve maximum quality and safety of the final cheese.

P2-13 Culture-Dependent and Independent Methods to Monitor the Evolution of Lactic Acid Bacterial Microflora in a Greek PDO Soft Cheese

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Introduction: "Katiki Domokou" is a traditional Greek soft cheese which has obtained the PDO recognition since 1994. It is a curd cheese, produced traditionally from goats' milk originating from a mountainous area in Central Greece. Lactic acid bacteria (LAB) constitute its main microflora but little is known concerning species and strains present.

Purpose: The purpose of this study was to elucidate the different species and strains of LAB and their evolution during storage at four different storage temperatures (5, 10, 15, 20°C).

Methods: Commercially available Katiki cheese was purchased and stored at four different temperatures (5, 10, 15, 20°C). Samples were taken at regular intervals. MRS and M17 media were used to determine the LAB population. Pulsed Field Gel Electrophoresis (PFGE) was used to monitor strain evolution and Denaturant Gradient Gel Electrophoresis (DGGE) in conjunction with 16S rRNA sequencing to identify the species present. HPLC was also applied to monitor the acids generated.

Results: The levels of the bacterial population recovered from MRS were stable throughout storage. On the contrary, the ones on M17 decreased during storage. The important finding of the present study was that the presumed starter culture (*Lactococcus lactis*) used in cheese manufacture was not recovered either from MRS or M17 medium, and it was only detected by application of DGGE. The majority of the isolates recovered belonged to different *Pediococcus* and *Lactobacillus* species. Regarding strain diversity, PFGE provided concrete results on how strain microflora is altered during storage. HPLC analysis demonstrated quantitative and qualitative differences of the metabolites generated.

Significance: The present study elucidated for the first time the microflora of a Greek PDO cheese consisting of strains probably belonging to an in-house microflora. "Safetywise" is a very important finding, since many pedlococcal strains have been reported to produce bacteriocins.

P2-14 Bacterial Dynamics in Model (Sterile) Portuguese Traditional Cheeses: A Case Study of Food Safety

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Introduction: Portuguese traditional cheeses are normally manufactured from small ruminants' raw milk. Hence, their native microflora — mostly Lactic Acid Bacteria (LAB) — as well as a few contaminating bacteria will undergo specific ecological evolution patterns throughout the ripening period. Such microbial population dynamics is partially responsible for several differences between cheeses.

Purpose: The purpose of this study was to understand the nature of microbial interactions that take place within the cheese matrix, knowing that absence of thermal processing of milk and manufacturing tools will eventually allow incorporation of spoilage/pathogenic bacteria.

Methods: Experimental cheeses were produced from sterilized raw ewe's milk, using plant rennet and following the traditional cheesemaking procedure: addition of salt, addition of rennet, incubation, cutting and whey removal. Unlike the procedure followed in traditional cheesemaking, milk was inoculated with either a single or a mixed culture, at a rate of 1% (v/v), previously obtained from regular cheeses. The mixed culture was composed of a combination of two bacteria, one LAB (i.e. *Lactococcus lactis*, *Lactobacillus plantarum* or *Lactobacillus brevis*) and one contaminating bacterium (i.e. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Listeria innocua*). The latter was added to milk, so as to reach three target levels in 0 d-model cheeses: 10^3–10^4, 10^5–10^6 and 10^7–10^8 CFU^{g-1}. Cheeses were kept up to 60 days at 7°C, to simulate the ripening environment. Viable cells and specific physico-chemical parameters were monitored throughout the ripening period.

Results: Distinct patterns of bacterial growth were observed, especially for cheeses inoculated with *L. lactis* in which the pathogens tested were not able to survive to 60 days. This may be due to the drop in pH since pH values around 4.60 were achieved by 2 days of ripening.

Significance: The presence of *L. lactis* as a part of the adventitious microflora is likely responsible for the increase in safety of traditional cheeses.

P2-15 Isolation, Identification, Virulence Tests and Determination of Pathotypes of *Escherichia coli* Isolated from Ricotta Cheese Commercialized in Campinas, São Paulo

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Introduction: Ricotta cheese is susceptible to microbial contamination by fecal coliforms including *Escherichia coli*, which has enteropathogenic strains that can express different virulence factors and cause extraintestinal diseases.

Purpose: Evaluating the microbiological quality of ricotta through isolation, identification, virulence factors and classifying pathotypes of *E. coli* isolated from it.

Methods: 32 samples of ricotta were inoculated into Petrifilm for counting and identification of *E. coli*. Confirmation was made by biochemical tests. One-hundred forty-two strains identified as *E. coli* were submitted to genotypic tests (PCR) for classifying in pathotypes and phenotypical tests.

Results: From 32 samples, 81.3% were contaminated with total coliforms being 59.4% of them by *E. coli*. From these, 137 strains (96.5%) showed some virulence factor, being 18 (12.95%) positive for some of the virulence genes tested. One strain (0.7%) presented the *stb* (heat-stable toxin) gene and 13 strains (9.1%) showed the *lt* (heat-labile toxin) gene indicating to be enterotoxigenic *E. coli* (ETEC). Four strains (2.8%) showed the gene CNF (cytotoxic necrotizing factor) being classified as extraintestinal *E. coli* (EXPEC) and one strain showed two genes (*eae* + *cnf*). Among phenotypical tests, the lecitinase was observed in 87.3% of the strains. Also, 86.6% showed hemolytic activity and 36 strains (25.35%) were characterized as having hemagglutinanation properties. From these, 8.3% presented as MSHA, while 72.2% presented as MRHA, which suggests the presence of distinct adhesin on the surface of their cells.

Significance: Strains of diarrheagenics (DEC) and extraintestinal (EXPEC) *E. coli* were found, so that Sanitary Vigilance should control inspection of ricotta to ensure food safety to population.

P2-16 The Prevalence of *Listeria monocytogenes* in Queso Fresco in Sinaloa, Mexico

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Introduction: The association of *Listeria monocytogenes* (*Lm*) outbreaks with Latin-style soft cheese has been well documented. The presence of *Lm* in fresh cheese, such as "Queso fresco" (QF), is a major public health concern in North, Central, and South America due to the popularity of this style of cheese. Since prevalence of *Lm* in QF and subsequent *Lm* outbreaks have been poorly documented in Central and South America, more information is needed to identify the niches of *Lm* in the food supply.

Purpose: The focus of this study was to identify the prevalence of *Lm* in QF in the northwestern Mexican state of Sinaloa.

Methods: We obtained a sample of QF from 75 independent merchants within Culiacan, the capital of Sinaloa, between the months of January and August of 2007. *Lm* was enriched in TSB and subsequently plated on Palcam Agar and *Listeria* ChroMagar. Isolates were verified as *Lm* using API, PFGE, and ribotyping.

Results: Sixteen isolates of *Lm* were found in 7 of 75 QF samples (9% sample prevalence, 1 to 5 isolates per sample testing positive for *Lm*). The results from PFGE molecular subtyping indicate that three different pulsotypes were found within these isolates. A majority of the isolates (14 of 16) belonged to pulsotype I, whereas pulsotypes II and III contained one isolate each.

Significance: The predominance of pulsotype I in QF samples obtained from Culiacan suggests two possibilities: 1) there is a common source of contamination for pulsotype I *Lm*, and/or 2) pulsotype I *Lm* thrives in the raw milk and /or process plant environment used for this region's QF. The relatively high prevalence of *Lm* in QF argues for research to identify the source of contamination, quantify levels and types of *Lm*, and develop intervention strategies.

P2-17 The Behavior of *Listeria monocytogenes* in Butter

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Introduction: Listeria monocytogenes can cause illness (listeriosis), which manifests itself in various ways, including septicemia, meningitis, and cervical infections that may result in abortion or stillbirth in pregnant women. Recent outbreaks of listeriosis have been linked to butter, a food which is traditionally regarded as "low risk".

Purpose: This study set out to determine why butter may be vulnerable to contamination, survival and/or growth of *Listeria monocytogenes*.

Methods: Different methods for inoculating *Listeria monocytogenes* into butter were investigated, to find one which best mimicked how butter might be contaminated during production and use. The impact of butter characteristics ('water droplet size', 'salt' and 'temperature') on the growth of *L. monocytogenes* was also investigated.

Results: A method ('mix') involving making a pit in the butter, adding a liquid inoculum, and gently blending, gave comparable results to a control ('cream') method in terms of *L. monocytogenes* behavior. Some butters were found to support the growth of *L. monocytogenes,* which is consistent with other workers' findings. Water droplet size, salt level and temperature of butter all had an impact on the growth rate and final level of *L. monocytogenes* in butter. The rate of growth was quickest, and the final level of counts highest, in butter with the coarsest structure (largest water droplet size). The more salt in the butter, the slower the growth rate of *L. monocytogenes*. Although slow, growth is still evident in butter with a total salt content of ~2% (~12% aqueous).

Significance: The findings underline the importance for butter manufacturers of considering *L. monocytogenes* in their food safety strategies. They show the importance of salt, good manufacturing technique and appropriate storage in preventing problems with *L. monocytogenes* growth.

P2-18 DSC Stability of Antibiotic Resistant *Enterococcus* sp. in the Dairy Fermentation Environment

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Introduction: Recently, antibiotic resistant (ART) commensal bacteria with mobile antibiotic resistance (AR) genes were found prevalent in retail foods, including dairy products, indicating that the food chain might have become a significant avenue in transmitting AR genes to humans. Revealing the mechanisms involved in the maintenance and dissemination of ART bacteria during cheese fermentation is essential for the development of targeted counter strategies to minimize contamination of processed dairy products with ART bacteria.

Poster

Purpose: The objective of this study was to characterize tetracycline resistance (Tet^r) *Enterococcus* sp. isolates from the cheese fermentation environment.

Methods: Tet^r bacteria isolated from Cheddar cheese made in the OSU dairy pilot plant were screened for the presence of *tet* genes by PCR, and AR gene carriers were identified by 16S rRNA gene sequence analysis. The AR gene-encoding genetic elements were characterized by Southern-blotting analysis, partial DNA sequencing, natural gene transformation and plasmid curing.

Results: Tet^r *Enterococcus* sp. isolates carrying *tetM* and *tetL* genes were found prevalent in the dairy fermentation environment. An enterococcal isolate was found carrying a novel 20-kb plasmid constructed with both *tetM* and *tetL* genes. The AR genes were very stable and were found functional and transferable to *Streptococcus mutans*.

Significance: The results suggested that the ART enterococci carried certain mechanisms to maintain the AR plasmid in the absence of antibiotic selective pressure and survive the dairy fermentation environment; revealing such mechanisms will help in developing counter control strategies.

P2-19 Microbiological Safety of Iru, Ogi and Kunuzaki; Three Small-Scale Fermented Foods Sold in Akure Metropolis, Nigeria

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Introduction: The microbiological safety of three locally fermented foods (iru, ogi and kunu-zaki) was investigated in this work.

Purpose: This survey was done to determine whether locally fermented foods sold were safe for consumption.

Methods: Ten different samples each of the fermented foods were collected at the Central Market (Oja Oba) and conventional methods were used to determine the numbers and types of bacteria and fungi present in the foods.

Results: The mean total bacterial count in the samples of iru, ogi and kunu-zaki were 3.3×10^7 CFU/g, 1.8×10^6 CFU/ml and 8.3×10^4 CFU/ml, respectively. Also, the mean total viable count of fungi was 1.9×10^6 CFU/g, 1.1×10^6 CFU/ml and 8.3×10^4 CFU/ml, respectively. Bacteria isolated from iru samples were *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp. and *Staphylococcus* sp. *Pediococcus* sp., *Leuconostoc* sp. and *Lactobacillus* sp. were isolated from ogi samples. *Lactobacillus* sp. was the only bacterium isolated from kunu-zaki. The fungi isolated from iru samples were *Passalora bacilligera*, *Penicillium* sp. and *Diplococcum spicatum*. *Saccharomyces cerevisiae*, *Fusarium* sp., *Candida* sp. and *Penicillium* sp. were isolated from ogi while *Rhizopus* sp., *Aspergillus flavus* and *Candida* sp. were isolated from kunu-zaki. It was noticed that ogi and kunu-zaki were bacteriologically safer for consumption than was iru because bacteria isolated from them were probiotics whereas *Bacillus* sp. and *Staphylococcus aureus*, which cause foodborne illnesses, were isolated from iru.

Significance: The work has shown that small-scale fermented foods in Nigeria are microbiologically safe to an extent, but the safety can be improved upon by implementing good hygienic practice, especially in the production of iru.

P2-20 Sensory Evaluation of Probiotic Yogurts and Fermented Milks with an Incomplete Balanced Paired Comparison Design

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Introduction: A wide range of probiotic bacteria can be used to formulate yogurts and fermented milks but their effect on sensory quality needs to be assessed. Paired comparison designs can be used in sensory tests for statistical analyses. Incomplete balanced blocks design (IBBD) is used when the comparisons between all studied treatments are equally important, so the combinations of the treatments used in each block will have to be selected in a balanced form.

Purpose: A sensory evaluation based on an IBBD was used to determine whether significant differences exist between acceptance of probiotic yogurts and fermented milks. IBBD is used when it is not possible to make all the combinations in each block due to limitations of the physical size of the block (in this study, 7 products).

Methods: Streptococcus thermophilus (ST), *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB), *L. acidophilus* (LA), *L. casei* (LC), and/or *L. reuteri* (LR) were used to manufacture a yogurt (ST+LB), three probiotic yogurts (ST+LB+LA, ST+LB+LC, ST+LB+LR) and three fermented milks (ST+LA, ST+LC, ST+LR). The test of preference used had a 4-point scale (1= like slightly more sample A than B, 2= like moderately more sample A than B, 3= like more sample A than B, and 0 = no preference) and was applied with 21 untrained judges to whom four samples were provided, a pair for the comparison and another pair to replicate the test (with different order of sample presentation).

Results: The analysis of variance revealed that there were no significant differences ($P > 0.05$) in preference between yogurts and fermented milks. By analyzing their comments on the evaluation form, judges based their scoring mainly on flavor (smoothness, intensity, and acidity) and consistency.

Significance: Scores given by the judges to products revealed that no differences existed in preference for probiotic yogurts and fermented milks containing *L. casei* or *L. acidophilus* or *L. reuteri*.

P2-21 Prevalence of *Salmonella* and *Listeria monocytogenes* in Raw, Bulk Tank Milk from United States Dairy Farms

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Introduction: Listeria monocytogenes and *Salmonella* are frequently isolated from dairy cattle and have been identified as bulk milk contaminants.

Purpose: The objective of this study was to determine the prevalence *of L. monocytogenes* and *Salmonella* in bulk milk from US dairy farms.

Methods: As part of the NAHMS Dairy 2007 survey, 541 milk samples and 523 in-line filters were collected from farms in 17 states and shipped overnight to the laboratory. Milk filters were pummeled with an equal weight of buffered peptone water. Filter extract and milk were enriched in selective broths. *Listeria* enrichments

were struck on modified Oxford medium. The presence of *Salmonella* was determined by real time PCR and positive enrichment broths were struck onto selective agar for isolation of *Salmonella*. Presumptive *Listeria* and *Salmonella* isolates were confirmed on several selective agars. *L. monocytogenes* was distinguished from other *Listeria* species via the presence of phosphatidylinositol-specific phospholipase.

Results: PCR analysis indicated that *Salmonella* was present in 13.9% of the milk samples and 33.3% of the filters. *Salmonella* isolates were obtained from 6.7% of the milk samples and 19.9% of the filters. Twenty-five *Salmonella* serotypes were identified, with the most common being Cerro (34), Kentucky (20), Muenster (14), Newport (10), Anatum (10), Montevideo (8), and Mbandaka (8). *Listeria* was detected in 7.8% of the milk samples and 26.8% of the filters while *L. monocytogenes* was detected in 4.3% of the milk samples and 6.5% of the filters.

Significance: Salmonella prevalence in bulk milk is higher than estimated in the NAHMS 2002 dairy survey (2.6% culture positive; 11.8% PCR positive). *L. monocytogenes* was more prevalent in the 2002 survey (6.5%). Milk filters were not collected during the 2002 study but results from the current study indicate that milk filter analysis is more sensitive for detecting the presence of *Salmonella* and *L. monocytogenes* than bulk milk analysis.

P2-22 Prevalence of *Escherichia coli* Virulence Factors in Raw Bulk Tank Milk from United States Dairy Farms

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Introduction: Cattle are known reservoirs of zoonotic pathogenic bacteria and several outbreaks of disease have been associated with the consumption of raw milk or raw milk products.

Purpose: A national survey of US dairies was conducted during the National Animal Health Monitoring System's (NAHMS) Dairy 2007 study to determine the prevalence of raw milk contaminated with pathogenic *Escherichia coli*.

Methods: Milk and inline milk filters were collected and shipped overnight to the laboratory. Milk was enriched in EC broth and milk filters were extracted in buffered peptone water prior to enrichment in EC broth. Multiplex real time PCR assays were used to determine the presence of the virulence genes *stx*1, *stx*2, *eae*A and ã-tir in the populations of organisms that grew in the enrichments.

Results: Shigatoxin genes (*stx*1 and/or *stx*2) were detected in 78 of 538 milk (14.5%) and 330 of 523 milk filter (63.1%) samples. The intimin gene (*eae*A) was found to be present in 104 milk and 388 milk filter samples and was found in association with one or both *stx* genes in 30 milk and 266 milk filter samples. Seven milk samples (1.3%) were found to contain a combination of *eae*A, ã-tir, and *stx*1 and/or *stx*2 that might indicate the presence of the highly pathogenic strain O157:H7, while such a combination was found in extracts from 78 filters (14.9%).

Significance: These data suggest that the incidence of detectable O157:H7 in bulk tank milk is low but there is the potential for contamination with other pathogenic forms. Since milk filters reflect what is entering the bulk tank, these data suggest the potential for a high level of contamination with pathogenic *E. coli*. Milk filter analysis appears to be a more sensitive test of milk contamination by pathogenic bacteria than bulk tank milk analysis.

P2-23 Extending Shelf Life by High Pressure Processing

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Introduction: Consumer demand for fresh, safe, shelf-stable products has paved the way for minimal processing technologies such as high pressure processing (HPP). Such non-thermal methods also allow development of shelf-stable products fortified with heat sensitive ingredients such as colostrum or vitamins. We evaluated product safety and stability in microbiological challenge trials with pressure processed product.

Purpose: To use HPP (a) to retain starter viability in commercial yogurt while selectively inactivating a dairy mold challenge, and (b) to optimize HPP conditions to extend the shelf life during ambient storage of an acidified (pH 4.0) milk beverage with heat-sensitive ingredients.

Methods:

(a) Commercial yogurt was inoculated at 10^6/ml with a dairy mold cocktail, then pressure processed at pressures from 360–500 MPa (without holding).

(b) Multiple strains of common milk flora contaminants were inoculated at 10^4 – 10^7/ml into pH 4 colostrum acid milk, then pressure processed at 600 MPa for 3 min. Microbial species used were: an eight species *Bacillus* cocktail; a five species *Lactobacillus* cocktail; a four species fungal cocktail; *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. Microbiological stability was evaluated in the HPP product during storage at 30°C/6 months.

Results: (a) In yogurt pressure processed above 460 MPa (without holding), molds were killed and starter viability retained. (b) HPP acid milk processed at 600 MPa/3 min reduced pathogenic and spoilage vegetative microflora to undetectable levels during storage at 30°C. Bacterial spores were not significantly reduced. Significantly, no spores germinated and grew in acid milk at pH 4.0 during six months storage at ambient temperature.

Significance: HPP treatment was effective in giving microbiological stability and safety in the acid dairy-based products studied.

P2-24 Effect of Mild Heat Treatment following High Pressure Processing on the Recovery of Pressure-Injured *Listeria monocytogenes* in Milk

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Introduction: Some studies have indicated the recovery of injured pathogenic and spoilage bacteria in nutrient broth, milk, and phosphate buffer after treatment by high pressure processing (HPP) followed by storage for 6 h to 4 weeks at various temperatures. The storage temperature after HPP would play an important role in bacterial recovery.

Purpose: The objective of this study was to develop a technique to inhibit bacterial recovery after HPP, focusing on the effect of post-HPP temperature treatment. In addition, we developed a probabilistic predictive model to optimize mild-heat treatment conditions to inhibit bacterial recovery after HPP.

Methods: Milk samples inoculated with *L. monocytogenes* (7 $\log_{10}$ CFU/ml) were treated by HPP (550 MPa for 5 min at 25°C) and then treated by various mild-heat treatments (30–50°C for 5–240 min). The number of *L. monocytogenes* cells was enumerated during storage after treatment. The mild-heat treatment conditions (temperature and holding time) required to inhibit the recovery of *L. monocytogenes* in milk was modeled using a logistic regression procedure.

Results: Immediately after HPP, no *L. monocytogenes* cells were detected in milk. However, the number of *L. monocytogenes* cells increased by > 8 $\log_{10}$ CFU/ml within 3 days storage at 25°C. Mild-heat treatment such as 50°C for 10 min inhibited the recovery of *L. monocytogenes* in milk after HPP during 70 days storage at 25°C. The mild-heat treatment conditions required to inhibit the recovery of *L. monocytogenes* in milk was modeled using a logistic regression. Percent concordance, which presents the ability to correctly predict outcomes (recovery/no recovery) was high (98.8%).

Significance: The predicted interface of recovery/no recovery can be used to calculate the mild-heat treatment condition to control bacterial recovery during storage at 25°C after HPP. The results in this study would contribute to enhanced safety of high-pressure-processed milk.

P2-25 DSC Phenotypic and Genotypic Analysis of *Staphylococcus* spp. from Raw Bovine Milk in Northeastern Brazil

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Introduction: Dairy production is very common in Northeastern Brazil. Milk and milk products could be very important sources of foodborne pathogens. In addition, antimicrobials used to treat udder infections may result in selection of resistant foodborne pathogens. In the study region, antibiotic usage is kept to a minimum, compared with usage in farms in developed countries. Other factors such as management and nutrition are also distinctly different.

Purpose: The purpose of this study was to determine prevalence, compare the antimicrobial resistance profiles, determine the occurrence of methicillin resistant *Staphylococcus* species, conduct molecular characterization using SCCmec-type, and investigate the clonality of *Staphylococcus* species using PFGE.

Methods: Staphylococcus isolation was done using conventional methods. Kirby-Bauer disk diffusion method was used to phenotypically characterize all the isolates for 10 antimicrobial agents. Multiplex PCR was used to genotypically characterize SCCmec-type (I-IV) and the detection of the mecA gene. PFGE was used to investigate the clonality of the isolates.

Results: More than half, 280 out of 552 (51%), of the total raw milk samples were positive for staphylococci. 59.6% were *S. aureus* and 46% Coagulase Negative staphylococci. About 16% were pan-susceptible and 36% were resistant to three or more antimicrobials. We detected phenotypic methicillin resistance among 102 (36%) of the isolates, but only 8 isolates carried the mecA gene. The most common antimicrobial resistance was to Ampicillin (43%). Twelve CoNS were resistant to Vancomycin. SCCmec-type I was the most common (15%) and 58% either did not carry SCCmec-type I-IV or did not have any SCCmec genes.

Significance: Staphylococcus isolates were common in milk. This could pose a significant food safety hazard to consumers. Methicillin resistance is present in whole milk. In addition, the identification of Vancomycin resistant staphylococci is of paramount significance, as VRSA is very rarely isolated worldwide.

P2-26 Alkaline Phosphatase Detection via Chemiluminescence in 45 Seconds with a One-Step Assay

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Introduction: Alkaline phosphatase is a heat sensitive enzyme in raw milk used as a thermal marker for pasteurization. The FDA and ISO approved methods for detecting phosphatase utilize fluorometric or chemiluminescent substrates that are photo-activated in the presence of this enzyme. US and EU regulations specify that pasteurized dairy products contain less than 350 mU/L alkaline phosphatase.

Purpose: To compare a newly developed chemiluminescent method, Fast Alkaline Phosphatase (FAP), to the approved methods.

Methods: The chemiluminescent method involves adding 100 ul sample to a pre-dispensed reagent vial. The mixture is inserted into a portable luminometer that monitors temperature. At 45 s, the chemiluminescence signal is converted into mU/L enzyme units. Luminometer calibration is programmed in a five min procedure using two tests of negative milk and a 350 mU/L calibration tablet. Cream and chocolate milk (pre-skimmed) use a 90 s analysis time. In the comparison study, lab pasteurized products were spiked with 0.002–0.5% raw bovine milk (or in the case of goat, 0.02%–5.0% raw goat milk) and tested by the FAP and by the approved methods.

Results: The FAP method replication is CV 10–15% in the range of 100 to 5000 mU/L enzyme in milk, skim, chocolate and goat milk. The accuracy of the method can be assessed by comparing the mean values determined by the three methods. In most levels of dairy matrices, the mU/L means were within 15–20% of the approved method means. These differences are within the expected repeatability (RSVr ~ 10%) of the approved methods, suggesting FAP is an equivalent measure of alkaline phosphatase in dairy products.

Significance: A convenient, rapid and portable testing system for pasteurization effectiveness promotes in-process plant testing by dairy personnel and provides earlier detection/confirmation of heat-time pasteurization deficiencies.

P2-27 Screening for Shigatoxin- and Intimin-Encoding Genes of Enterohemorrhagic *Escherichia coli* and for *Salmonella* on Cattle Carcasses and Beef Products

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) strains on raw or insufficiently cooked foods are of public health concern, as serious disease may

result from their ingestion. Therefore, many commercial producers of beef products screen for these pathogenic bacteria (especially *E. coli* O157:H7) prior to shipment, and while *Salmonella* is not considered an adulterant on raw beef products, it is used as an indication of process control.

Purpose: During 2005, we tested several commercial meat processing plants to determine the fraction of carcasses and lots of beef products that were contaminated with EHEC and/or *Salmonella*.

Methods: After a brief cultural enrichment, the polymerase chain reaction (PCR) was used to detect lots of beef products presumptively positive for bacteria carrying the Shiga toxin genes (*stx*1 and/or *stx*2), the attaching and effacing gene (*eae*) encoding intimin, and for *Salmonella*.

Results: Of 124,245 lots sampled, 9.93% were positive for the *stx*1 and/or *stx*2 genes, 7.60% for the *eae* gene and 2.52% for *Salmonella* by the PCR assay. Generally, lots of ground beef showed the lowest frequency of contamination and variety meats the highest. Leaner products tended to have lower frequencies of positive samples than those with higher fat content. Of all the samples, 3.66% contained EHEC (Positive for the *eae* gene and the *stx*1 and/or *stx*2 genes) and of those positive samples, 22.4% were also positive for *Salmonella*.

Significance: Contamination of carcasses and meat products by EHEC and *Salmonella* are apparently not independent occurrences. It appears that controlling the prevalence of one will also reduce the prevalence of the other. Interestingly, leaner meat had a lower prevalence of contamination by these pathogens. Such information should be useful in designing risk-based sampling protocols for meat processors.

P2-28 Influence of Lot Size and Sampling Procedures on the Incidence of *Escherichia coli* O157:H7 Detection in Meat Trim

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Introduction: Appropriate sampling protocols are essential to accurately estimate the potential contamination of food products. Food Safety Inspection Service (FSIS) has established a sampling protocol (FSIS N = 60 protocol) that considers the potential sources of contamination, typical process control, and product physical characteristics of the processed meat products.

Purpose: The purpose of this study was to compare the results of microbiological analyses of meat trim samples with the standard sampling procedure, FSIS N = 60 (5 combos) with a sampling protocol in which N = 60 (1 combo) samples were obtained using a coring device that sampled the meat trim at multiple depths, coupled with a more rigorous criterion for lot acceptance or rejection.

Methods: Sample collection and compositing of the meat from a lot size (one to five combos per lot) in processing plants were carried out by: 1) Hand trimming with standard FSIS N = 60 and 2) Core sampling with N = 60 from single combo. Enrichment cultures of the meat samples (in tryptic soy broth for a minimum of 8 h at 42 ± 2°C) were composited and analyzed for the presence of *E. coli* O157:H7 by multiplex PCR. Reactions generating appropriately-sized amplicons were scored positive for *E. coli* O157:H7. The *E. coli* O157:H7 incidence rates between the sampling procedures were compared by use of the Chi-square test for independence.

Results: This study demonstrated that mechanical core sampling and N = 60 combo size method yielded a significant ($P < 0.001$) thirty-fold increase in the number of lots confirmed positive for *E. coli* O157:H7, when compared to the number of positive lots using the standard FSIS N = 60 protocol.

Significance: The results in this study indicate that the alternative sampling procedure led to a significantly higher incidence of *E. coli* O157:H7 detected in the meat lots and thus a more accurate estimation of the public health risk status of the meat trim.

P2-29 Recovery Efficiency of Total Viable Counts from Beef Carcasses with the Surface Sponge Sampling Method

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Introduction: The safety and quality of beef products are commonly assessed by measurement of Total Viable Counts (TVC) using the surface sponge sampling method. Normally, this involves a single sponging of one to three 100 cm^2 areas on the carcass surface. However, there is inadequate information about the quantity of TVC recovered from the beef carcass surface using such a standard procedure. Understanding the efficiency of TVC recovery can lead to improved methods with lower test variability.

Purpose: The purpose of this study was to determine the efficiency of an internationally accepted meat sponge sampling method on the recovery of TVC from chilled beef carcasses.

Methods: Polyurethane sponges were used to sample 100 cm^2 areas on chilled beef carcasses as detailed in guidelines of the Australian Standard for production and transportation of meat and meat products for human consumption. Each 100 cm^2 area (n = 10) was sponged with five individual sponges and the sampling area excised. The TVC in sponges and on excised tissue were enumerated using APC Petrifilm incubated at 25°C for 72 h.

Results: Analysis of total TVC recovered from spongings plus the excised tissue showed that an average of 32 (s.d. = 23%), 48 (s.d. = 24%), 55 (s.d. = 24%), 62 (s.d. = 23%) and 68% (s.d. = 23%) of TVC were recovered with the 1st, 2nd, 3rd, 4th and 5th spongings, respectively. For the 10 carcass sites tested, the five total spongings removed an average of 68% (s.d. = 23%) of the total recoverable TVC (sponges plus tissue), with a range of 11 to 97%.

Significance: These data demonstrate that a single sponging of a 100 cm^2 area recovers, on average, less than 50% of the total recoverable TVC. The products of this research reduce the uncertainty of TVC measurements among different databases and provide a more accurate interpretation of factors that influence the microbiological quality of meat products.

P2-30 Prevalence and Distribution of *Escherichia coli* O157 on Beef Carcasses at Three Slaughter Plants

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Introduction: In recent years the number of recalls due to *Escherichia coli* O157 in the beef industry had declined. However, in 2007 the number increased, and research is needed to determine how the contamination is getting into the ground products and how to control it.

Purpose: The purpose of this study was to determine the pattern of *E. coli* O157 contamination on beef carcasses in commercial processing facilities across the United States.

Methods: Three slaughter plants in geographically distinct areas of the US were sampled, using a sterile hydrated spongecicle, for each season over the course of a year. A total of four sampling locations on the carcass were sampled, as well as the hides. Each carcass sampling location was sampled at three different locations in the plant, pre-evisceration, before interventions and after interventions. All samples were analyzed to detect and quantify the amount of *E. coli* O157 on the carcass and hide locations.

Results: A total of 51 of 3570 (1.43%) samples collected on the carcasses were positive for *E. coli* O157and 55 of 290 (18.97%) hide samples were positive. Of all the pre-evisceration carcass sampling locations, the hindshank was the most contaminated, with 7.93% of the samples testing positive, followed by the inside round with 4.48%. Very few positive samples (< 1%) were recovered from the foreshank and midline. There is significant plant to plant variation, with one plant having 42.5% of hindshanks and 27.5% of inside rounds testing positive for the pathogen. Additionally, at this plant, the incoming hide contamination for *E. coli* O157 was at 35%.

Significance: Identifying patterns of contamination in processing plants will enable the implementation of interventions that target high contamination areas so as to result in further reduction of the pathogen in the beef supply.

P2-31 DSC Survival of *Escherichia coli* O157:H7 in Ground Beef Following Sublethal Heat-Shock and Subsequent Isothermal Cooking

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Introduction: It has been suggested that sublethal heat-shock conditions during slow-cooking may enhance survival of *Escherichia coli* O157:H7 and jeopardize safety of slow-cooked products such as beef roasts. Studies have shown that heat-shock of *E. coli* O157:H7 in a broth medium leads to varying survival during subsequent heating in broth medium or ground beef slurry. Different heat-shock conditions have been reported as leading to the greatest heat-resistance in *E. coli* O157:H7.

Purpose: In this study, we examined the effect of heat-shock treatments of lean (6–9%) ground beef on the survival of *E. coli* O157:H7 in the same ground beef during a subsequent 4-h 54.4°C simulated cooking process.

Methods: Six different combinations of heat-shock temperature (47.2, 48.3, and 49.4°C) and time (5 and 30 min) were applied to a 5-strain cocktail of anaerobically-grown cells in 25 g of pre-warmed ground beef, followed by heating at 54.4°C for 4 h. In order to enumerate viable uninjured and injured cells, samples were spread-plated on nutrient agar, incubated for 1 h at 35°C, and then overlaid with a selective medium.

Results: Temperature during a 30-min heat shock treatment did not significantly affect *E. coli* O157:H7 survival on subsequent isothermal cooking ($P > 0.05$). Survival following a 5-min heat-shock was higher when the heat-shock temperature was 48.3 or 49.4°C ($P < 0.05$) than when it was 47.2°C. Mean (n = 3 trials) reductions in *E. coli* O157:H7 during the 4-h 54.4°C isothermal cooking process ranged from 4.0 to 7.7 log CFU/g. Compared to results in model systems (broth or slurry), these results in ground beef are more likely to be representative of pathogen survival during a slow-cook process.

Significance: Our results indicate that heating in the temperature range of 48–50°C for 5 min may increase survival of *E. coli* O157:H7 during a subsequent slow-cooking process.

P2-32 Survival of Non-O157:H7 STEC on Beef Tissue Surface Following Spray Treatment with Different Antimicrobials

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Introduction: Non-O157 shiga toxin producing *Escherichia coli* (non-O157 STEC) are a significant public health issue based on increases in human illness involving these organisms. Limited published data are available on the effectiveness of antimicrobial treatments against these pathogens when applied to meat surfaces.

Purpose: The purpose of this study was to evaluate the efficacy of three carcass treatment antimicrobials against four non-O157 STEC strains and one *E. coli* O157:H7 strain at high and low inoculum levels.

Methods: Antimicrobials containing the following actives were used: acidified sodium chlorite (1000 ppm), octanoic acid (9000 ppm) and peracetic acid (200 ppm). *E. coli* isolates, O157:H7, O145, O111, O26:NM and O26:H11, were grown in TSB (35°C/24 h) then diluted to targeted levels of 10^6 and 10^3 CFU/sample. Each diluted isolate was inoculated (50 µl) onto a single piece of meat (25 cm^2). An attachment time of 30 min was allowed, followed by an antimicrobial treatment spray for 15 s. Samples were held at 2–8°C for 3 h, neutralized with DE Broth, and then sampled for survivors by direct plating on Sorbitol MacConkey Agar (35°C/24 h). The study was performed in triplicate and sterile water was used as a treatment control.

Results: No difference ($P > 0.05$) in efficacy was observed between strains for each treatment. Population reductions in each treatment type for each non-O157 STEC showed between 0.74 and 1.12 log CFU/sample for low inoculum and between 0.68 and 1.63 log CFU/sample for high inoculum, while reductions in *E. coli* O157:H7 populations were between 0.55 and 1.88 log CFU/sample for both inoculum levels.

Significance: These data suggest that non-O157 STEC and *E. coli* O157:H7 are of equivalent sensitivity to each antimicrobial active used in this study and that therefore the level of control supplied should be no different.

P2-33 Impact of Slicer Design and Blade Type on Quantitative Transfer of *Listeria monocytogenes* during Slicing of Ham

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Introduction: Listeria monocytogenes isolation rates have remained seven times higher for delicatessen-sliced than for manufacturer-sliced deli meats, with adequate cleaning/sanitizing of delicatessen slicers still problematic because of design constraints.

Purpose: This study examined two commercial slicers — a Hobart (model 2612) and Berkel (model X13) slicer, and three different slicer blades for numbers of *L. monocytogenes* transferred during ham slicing.

Methods: Three commercial slicer blades (2 Hobart, 1 Berkel) were surface-inoculated with a 6-strain *L. monocytogenes* cocktail (~10^3 CFU/blade) by slicing a previously surface-inoculated ham chub and were then used to slice uninoculated ham to obtain 100 slices. The first 25 slices were individually diluted 1:5 in University of Vermont Medium, homogenized, pour-plated (10 ml) in 25 ml of Modified Oxford Agar and also enriched for *Listeria*. Slices 26 through 100 were consecutively composited into15 sets of 5 slices each, stored at 4°C for 5 days and then examined for *Listeria* as just described. Each experiment was replicated six times. After slicing, nine different locations on each of the two slicers were also assessed for numbers of *Listeria*, using our 1-ply composite tissue sampling method.

Results: Hobart blade 1 yielded greater *Listeria* transfer ($P < 0.05$) than Hobart blade 2 and the Berkel blade, with no significant difference between the latter two for slice 1 through 25. However, for slices 26 through 100, the Berkel blade yielded greater *Listeria* transfer ($P < 0.05$) than Hobart blades 1 and 2. In addition, Hobart blade 2 transferred significantly fewer ($P < 0.05$) listeriae to the collection area, back plate, guard front, and guard holder of the slicer than Hobart blade 1 and the Berkel blade.

Significance: In addition to ease of cleaning and sanitizing, these findings indicate, slicer and blade design must be considered when developing slicers for minimal bacterial transfer.

P2-34 *Listeria monocytogenes* Growth in Delicatessen Meats Based on Product Formulation, Age and Temperature

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Introduction: Delicatessen meats remain the leading cause of foodborne listeriosis in the United States, with growth of *Listeria monocytogenes* varying based on product formulation and storage temperature.

Purpose: The impact of product formulation, age and temperature on growth of *Listeria* was examined to develop future recommendations for "best consumed by" dating of deli meats.

Methods: Six retail brands each of cured turkey breast, uncured turkey breast, roast beef and ham (3 lots/brand) with/without *Listeria* growth inhibitors were sliced and surface-inoculated with an 3-strain cocktail of *L. monocytogenes* (~40 CFU/g) when received and again at the midpoint and last date of sale. All inoculated and uninoculated control samples were analyzed for numbers of *L. monocytogenes* and background flora during extended storage at 4, 7, and 10°C by plating on Modified Oxford Agar and Trypticase Soy Agar containing 0.6% yeast extract, respectively.

Results: L. monocytogenes grew faster in deli meats without lactate and/or diacetate than in those with inhibitors. *Listeria* inhibitors in the different products showed varying degrees of effectiveness ranging from total to only partial inhibition. For products supporting growth, typical *L. monocytogenes* generation times were 0.4, 0.7, and 1.1 days at 10, 7 and 4°C, respectively in brands without *Listeria* inhibitors, and 0.7, 1.7 and 3.3 days at 10, 7 and 4°C, respectively, when *Listeria* inhibitors were present. Curing agents, such as nitrite, in the turkey breast (both without lactate and/or diacetate) did not inhibit growth of *L. monocytogenes*. Significant differences ($P < 0.05$) in *L. monocytogenes* growth were seen between different lot numbers of the same product, with *L. monocytogenes* growth strongly influenced by the levels of background microflora.

Significance: These findings stress the importance of *Listeria* inhibitors, background microflora and temperature when developing scientifically based "best consumed by" dates for deli meats.

P2-35 Control of *Clostridium perfringens* Germination and Outgrowth in "Natural" Turkey Roast by Lemon Juice Concentrate and Vinegar Mixtures

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Introduction: Increasing consumer demand for minimally processed, natural meat products have resulted in greater numbers of such products in the market. Several of the traditionally used antimicrobial ingredients are not permitted for use in "natural" Ready-to-Eat (RTE) meat and poultry products. Thus, there is a need to assess the safety and evaluate compatible antimicrobial agents for use in such products.

Purpose: Spores of foodborne pathogens can survive and potentially germinate and grow in thermally processed RTE meat and poultry products if these products are not properly cooled. The goal of the study was to evaluate inhibition of germination and outgrowth of *C. perfringens* spores by antimicrobial ingredients compatible for use in natural poultry products during abusive cooling of RTE turkey product.

Methods: A traditional formulation of turkey roast containing turkey breast meat, sea salt and turbinado sugar was used. Antimicrobial ingredients containing lemon juice concentrate and vinegar (MoStatin and MoStatin LV) were incorporated into the product at various concentrations. The ingredients were mixed and portions of the product were transferred to plastic bags. The product was inoculated with *C. perfringens* spores, mixed, vacuum packaged, heat shocked for 20 min at 75°C and chilled exponentially from 54.5 to 7.2°C in 6.5, 9, 12, 15, 18 or 21 h.

Results: C. perfringens was able to grow in control samples from an initial population of ca. 2.3 log CFU/g by 1.3, 3.3, 4.5, 5.8, 6.6 and 6.9 log CFU/g subsequent to 6.5, 9, 12, 15, 18 and 21 h exponential chill times, respectively. Addition of antimicrobials (MoStatin and MoStatin LV) inhibited germination and outgrowth of *C. perfringens*.

Significance: Microbiological safety of the "natural" RTE meat and poultry products should be evaluated and antimicrobial ingredients compatible with "natural" RTE meat and poultry products should be incorporated to assure their safety. These findings will be of immediate use to the retail food service operations and regulatory agencies to guard against the hazards associated with *C. perfringens*.

P2-36 Reduction of *Salmonella* in Ground Turkey and Turkey Breasts with a *Lactobacillus*-Based Intervention

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Introduction: Lactic acid bacteria (LAB) are inhibitory against various pathogenic bacteria during growth and storage of ground meat samples.

Purpose: The effectiveness of a combination of 4 strains of *Lactobacillus* spp. in reducing *Salmonella* in turkey products was evaluated to improve the final safety of the product.

Methods: Turkey breasts were inoculated with a 3 strain *Salmonella* cocktail and then treated with a 1 × 10^6 CFU/ cm^2 of a *Lactobacillus* based intervention made up of NP51, NP3, and NP7. The turkey breasts were stored at either 5°C and sampled at days 0, 1, 2, and 3 or at 37°C and sampled at h 0, 2, and 4. Ground turkey was inoculated with a 3 strain *Salmonella* cocktail and then treated with a 1 × 10^6 CFU/ cm^2 of a *Lactobacillus* based intervention made up of NP51, NP3, and NP7. The ground turkey was stored at 5°C and sampled at days 0, 1, 2, and 3.

Results: The turkey breasts stored at 5°C treated with *Lactobacillus* had a 2 log ($P < 0.05$) reduction of *Salmonella*. *Salmonella* in the turkey breasts kept at 37°C treated with *Lactobacillus* showed a 1.5 log ($P < 0.05$) reduction by h 2 and a 2 log ($P < 0.05$) reduction of *Salmonella* by *h* 4. *Salmonella* in the ground turkey kept at 5°C treated with *Lactobacillus* showed a 2 log ($P < 0.05$) reduction.

Significance: These results show that the addition of *Lactobacillus* to turkey products significantly reduces *Salmonella* during storage.

P2-37 Prevalence and Level Distribution of *Salmonella* spp. from Retail Pork Cuts from Four United States Cities

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Introduction: The aim of this study was to establish a distribution of *Salmonella* in retail pork chops and roasts, for development of a quantitative risk assessment to determine risks associated with consumer cooking and consumption of pork products, Here, samples from retail establishments in four US cities were collected and assayed for *Salmonella* prevalence and levels.

Methods: Four product types, including unenhanced and enhanced pork chops and unenhanced and enhanced boneless pork roasts, were collected at retail establishments in Dallas, TX, Green Bay, WI, Phoenix, AZ, and San Antonio, TX. Initially, equal sample numbers were collected from each area and product type. However, subsequent sampling targeted Phoenix and enhanced products, which demonstrated higher positive frequencies. Composited enrichments were screened for *Salmonella,* using PCR-BAX®. Presumptive positive composites were then re-sampled and similarly tested individually. Those presumptive positives were re-sampled and then confirmed, following USDA FSIS methods, and enumerated by MPN. Four thousand pork samples were assayed from January to April, 2007. FSIS prevalence data were used to estimate the number of samples needed to estimate the 90th percentile of the distribution of *Salmonella* on pork.

Results: Salmonella was confirmed in 28 samples, including: 1.47, 0.31, 0.13, and 0% of retail pork samples from Phoenix, Green Bay, Dallas, and San Antonio, respectively. *Salmonella* was isolated from 18 of 1,350 enhanced pork chops (1.33%) and 10 of 1,350 enhanced boneless pork roasts (0.74%), but undetected in 1300 unenhanced products; Most positive samples (15; 53.6%) were below the MPN detection limit (< 0.30 MPN/g), but 13 samples ranged from 0.30 to 1.40 MPN/g.

Significance: The study confirmed 28 positives of 4000 pork samples (0.70%), yielding a *Salmonella* distribution for use in an exposure assessment. The study also demonstrated that *Salmonella* frequency and levels are low in retail pork, but varied by region and processing type.

P2-38 Minimum Nitrite Level Required to Inhibit *Listeria monocytogenes* on Ready-to-Eat Turkey Prepared with Lactate and Diacetate

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Introduction: Lactate and diacetate are more effective in inhibiting *Listeria monocytogenes* (*Lm*) in cured Ready-to-Eat (RTE) meats than in uncured products, but no study has identified the threshold level of nitrite required to prevent *Lm* growth in these products.

Purpose: The objective of this project was to compare the antilisterial effect of various nitrite levels in RTE sliced turkey product manufactured with lactate and diacetate.

Methods: Treatments were manufactured using a central composite design for 4 variables: sodium nitrite, sodium chloride, potassium lactate, and sodium diacetate, with 5 levels for each variable (total 30 runs; center point replicated 5 times). Ranges for antimicrobial testing included 0–120 ppm nitrite, 0.8–3.6% NaCl, 0–3.2% lactate, and 0-0.24% diacetate. Sliced finished products were surface inoculated with 3-log CFU/g *Lm*, vacuum-packaged, stored at 4°C, and assayed for changes in populations of *Lm* for up to 18 weeks.

Results: Listerial growth (defined as a 1-log increase) was highly variable for samples formulated with 30 and 60 ppm nitrite. Growth for center point treatment samples formulated with 60 ppm was observed between 6 and 18 weeks at 4°C (compared to 13 weeks predicted by a commercially available model using 100 ppm nitrite). Formulations with similar lactate-diacetate-NaCl combinations supported growth at 15–18 weeks for 120 ppm nitrite and 3–6 weeks for no nitrite. Treatments with other combinations of lactate-diacetate-NaCl and 90 ppm nitrite supported growth similar to that which was predicted by the commercial model. Inhibition of *Lm* was decreased by 3–6 weeks in several treatments with 30 ppm nitrite compared with the predictive model, but delayed growth by 3 weeks or more compared to the control without nitrite.

Significance: These results suggest that a minimum 30 ppm nitrite will enhance the antilisterial activity of lactate-diacetate in RTE poultry, but as with other traditional antimicrobials, the effect is concentration dependent.

P2-39 DSC Efficacy of Chlorine Dioxide against *Listeria monocytogenes* in Brine Solutions

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Introduction: Chilled brines are employed by the food industry to rapidly cool Ready-to-Eat meat products after cooking and before packaging.

Poster

Purpose: Antimicrobials, such as chlorine dioxide (ClO_2), are being investigated to inhibit *Listeria monocytogenes*, which has the potential to contaminate and survive in brines.

Methods: Several experiments were performed using brines made of calcium chloride ($CaCl_2$) and sodium chloride (NaCl), inoculated with *L. monocytogenes*, and/or treated with 3 ppm ClO_2. Ten and 20% $CaCl_2$ and NaCl solutions (pH 7.0) were inoculated with a 4-strain cocktail of *L. monocytogenes* to obtain ~7 $\log_{10}$ CFU/ml and incubated 8 h at 4°C. Since ClO_2 could not be measured in strong brines, it was measured indirectly in deionized water (Photometer Chlordioxmeter 1000).

Results: The results demonstrated that *L. monocytogenes* survived in 10% $CaCl_2$ and 10 and 20% NaCl. However, *L. monocytogenes* was reduced ~1.2 $\log_{10}$ CFU/ml in 20% $CaCl_2$. Inoculated (~7 $\log_{10}$ CFU/ml) brine solutions (10 and 20% NaCl, and 10% $CaCl_2$) treated with 3 ppm ClO_2 resulted in a ~4 $\log_{10}$ reduction of the pathogen within 90 s. However, ~3 ppm ClO_2 in 20% $CaCl_2$ appeared to have a protective effect against *L. monocytogenes*, since a ~2 log reduction was observed within 90 s. Additional experiments demonstrated that 20% $CaCl_2$ affects the activity of 3 ppm ClO_2 by potentially binding the compound, interfering with activity, or diminishing the oxidizing capacity. Finally, spent brines from hot dog and ham processing were treated with ClO_2 at concentrations of 3 or 30 ppm. At these concentrations, ClO_2 did not reduce *L. monocytogenes*, presumably because of the presence of organic material.

Significance: Therefore, reduction of organic material in spent brines prior to disinfection with ClO_2 should be investigated to improve the efficacy of the compound against *L. monocytogenes* under the conditions described.

P2-40 Evaluation of Spraying a Lactic Acid Based Antimicrobial Product on the Reduction of *Salmonella* on Broiler Chickens

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Introduction: Controlling *Salmonella* cross contamination during processing is a challenge for the broiler industry. Thus, new and cost-effective intervention techniques to reduce the *Salmonella* during processing are needed.

Purpose: The objectives of this study were to determine the effect of the lactic acid-based antimicrobial product Chixide® in reducing *Salmonella* during processing on broiler chickens.

Methods: A total of 25 broilers were raised and slaughtered at the Texas Tech University poultry processing facility. Following evisceration (30 min PM) the carcasses were inoculated with a 10^6 CFU/ml cocktail of *Salmonella* by placing the *Salmonella* cocktail with 400 ml of BPW into a poultry rinse bag and shaking the solution with the bird for 1 min. Following a 30-min attachment period, treatments were applied by using the lactic acid product or sterilized water (control). The applications were a 5-s spray, a 5-s dip, a 10-s dip, and a 20-s dip. Following treatment, each carcass was placed immediately into a carcass rinse bag with 400 ml of BPD according to USDA Whole Bird Rinse Guidelines and shaken for 1 min. Serial dilutions were plated onto XLT4 plates with a thin Tryptic Soy Agar overlay to allow for injured cell recovery. The XLT4 plates were incubated at 37°C and read after 48 h.

Results: There were no significant differences in inoculation levels of *Salmonella* on the carcasses prior to treatment application. Spraying the carcasses with the antimicrobial for 5 s yielded a significant *Salmonella* reduction of 1.3 log CFU/ml, while dipping the carcasses for 5, 10, or 20 s all yielded a 2.3 log CFU/ml reduction.

Significance: Based upon the results of this experiment, dipping broiler carcasses into the antimicrobial for 5 s reduced *Salmonella* significantly. However, using the spray method in a poultry processing environment does reduce *Salmonella* significantly by 1.3 log/ml, and this method could likely be implemented more easily into current processing environments and would be beneficial in improving food safety.

P2-41 DSC Evaluation of the Reduction of *Escherichia coli* O157:H7 and *Salmonella* spp. by Spraying a Lactic Acid-Based Antimicrobial Product on USDA Select Beef Tips

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Introduction: A newly developed, commercially available spray solution that can be applied in beef processing plants as a final intervention step prior to chilling or freezing was evaluated. According to company trials, this spray solution reduces *Escherichia coli* O157 and *Salmonella* on meat products. However, it has not been validated independently.

Purpose: The objective of this study was to validate the effectiveness of this lactic acid based antimicrobial product in reducing *Escherichia coli* O157:H7 and *Salmonella* on beef tips.

Methods: USDA Select beef tips were inoculated with either a cocktail mixture of *E. coli* O157:H7 or *Salmonella* at 10^5 CFU/ml. After 30 min of attachment, each beef tip was placed into a spray cabinet and either the lactic acid based antimicrobial product (Beefxide®) or sterilized water (Control) was sprayed at one foot per 2.5 s chain speed. The external surface of each beef tip was swabbed (100 cm^2 area) to determine pathogen load. The samples were plated onto aerobic plate count agar (APC) and either MacConkey agar (MAC) for *E. coli* O157:H7 or xylose lysine desoxycholate agar (XLD) for *Salmonella*. The data was analyzed by use of a descriptive analysis in SAS.

Results: Prior to inoculation, the beef tips had an initial aerobic plate count of 3.5 log CFU/100 cm^2 and a generic *E. coli* count of 1.5 log CFU/100 cm^2. After the beef tips were treated with the antimicrobial, the aerobic plate counts decreased by 1.5 logs ($P < 0.05$). The *E. coli* O157:H7 was reduced by 1.4 logs ($P < 0.05$) and the *Salmonella* sp. by 1.1 logs ($P < 0.05$), compared to the control samples.

Significance: These results prove that the antimicrobial is an effective spray intervention for reducing *E. coli* O157:H7 and *Salmonella* on meat cuts.

Poster

P2-42 HACCP Validation for Use of Lactic Acid on Bologna, Ham, and Red Hot Ready-to-Eat Meat and Poultry Products

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Introduction: The frequent incidence of *Listeria monocytogenes* (*Lm*) in Ready-to-Eat (RTE) meat and poultry products led to a USDA / FSIS final rule for the post-processing lethality control of *Lm* (9 CFR 430). RTE meat and poultry products processing plants must include control programs for *Lm* in their HACCP plans and verify their effectiveness against *Lm*.

Purpose: The objective of this study was to evaluate 2% lactic acid (LA) for its effect as a post-lethality treatment and antimicrobial inhibitor on the survival of *Lm* on RTE meat and poultry products produced by a Southeastern meat manufacturing company.

Methods: Red Hots (miniature frankfurter), Bologna, and Souse Roll samples were provided by the manufacturer. Samples were inoculated with approximately 7 log CFU/ml of *Lm* in 0.1% peptone water at 25°C for 20 s *Lm* recovery and enumeration after direct plating on PALCAM and/or USDA enrichment (when no growth) from samples after inoculation was in the range of 4–6 log CFU/g depending on size and type of product. Half of the inoculated samples were surface sprayed with 2% LA for 20 s and the other half kept as controls. All samples were individually placed in vacuum-sealed bags and stored at 4°C (three replicates) for 0, 7, 30, 60, and 90 days.

Results: Surface treatment of RTE meat and poultry products by 2% LA caused a significant reduction ($P < 0.01$) immediately after treatment (day 0) in the initial *Lm* counts by ≥ 1 log CFU/g compared to the controls. After 7 days storage, *Lm* counts were reduced by ≥ 2 log CFU/g in all products. *Lm* counts had decreased to undetectable levels in Souse Roll and Red Hots after 60 days with 2% LA treatment but *Lm* in Bologna increased after 7 d and remained at ≥ 1 log reduction from initial control (untreated levels).

Significance: The effect of 2% LA on *Lm* counts differed according to meat type and time of storage; however, ≥ 1 log CFU/g reduction was achieved with application of all three products.

P2-43 Survival of *Campylobacter jejuni* on Sterile and Naturally Contaminated Vacuum-Packed Beef and Pork at Refrigerated Temperatures

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Introduction: Campylobacter jejuni does not grow at temperatures below 28°C and is intolerant of aerobic conditions. Therefore, its survival, rather than growth, on meat must be a major factor affecting the incidence of campylobacterosis.

Purpose: Studies of *C. jejuni* survival on meat are few. The present study examined the survival of *C. jejuni* on sterile and naturally contaminated beef and pork stored at refrigerated temperature and vacuum packed.

Methods: C. jejuni NCTC 11168 was inoculated at 5–6 log CFU/25 cm² sterile cores of beef and pork, packed under vacuum and stored, with uninoculated controls, for 42 days at -1.5°C and 4°C. Simultaneously, naturally contaminated beef and pork loins were inoculated with *C. jejuni* at 5–6 log CFU/g and stored under similar conditions. At predetermined intervals, the numbers of surviving *Campylobacter* on inoculated cores were determined by direct plating on Tryptic Soy Agar and culturable *Campylobacter* were recovered by enrichment followed by plating on CCDA. Changes in the natural meat microflora were enumerated by plating on selective and non-selective media.

Results: Numbers of *C. jejuni* inoculated on sterile beef and pork cores dropped to 1 log unit within 7 days of storage and were undetectable at day 9 by direct plating. However, *C. jejuni* was detected up to day 28 following enrichment. On naturally contaminated beef and pork, *C. jejuni* could be recovered by direct plating for the entire storage time and the total population of *C. jejuni* dropped by a maximum of 3 log CFU/g. No abnormal change in the natural meat microflora was observed.

Significance: The results indicate that *C. jejuni* can survive longer on meat at refrigeration temperatures in the presence of the natural microflora than when it is alone on otherwise sterile meat.

P2-44 DSC Effectiveness of Non-Thermal Atmospheric Plasma on Reducing Foodborne Pathogens on Raw Poultry

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Introduction: The presence of *Salmonella* on raw poultry continues to be a problem. Additionally, research indicates that consumers continue to practice unsafe handling of raw poultry. Non-thermal atmospheric pressure plasma holds the potential to serve as a cold pasteurization step to minimize or eliminate *Salmonella* and other pathogens on poultry. This type of plasma works by creating energetic particles from molecules in the atmosphere and bringing them to the surface of the tissue.

Purpose: This research investigated the effectiveness of plasma to reduce *Salmonella* on media and raw chicken.

Methods: A multi-antibiotic resistant strain of *Salmonella enterica* (ATTC # 19214, resistant to tetracycline, chloramphenicol, and streptomycin) and a control strain (ATCC #13076) were identified. The sensitivity of the strains to plasma was evaluated by inoculating the surface of agar with the strains at levels from 10^1–10^6. Plates were exposed to plasma for multiple time periods. Effectiveness was determined by presence or absence of growth following incubation at 37°C for 48 h. The antibiotic resistant *Salmonella* was used to evaluate the effectiveness of plasma on raw chicken breast. Breasts were inoculated with approximately 1×10^6 *Salmonella* and the inoculated surfaces were exposed to plasma for a range of times.

Results: The two strains showed equal sensitivity to plasma, with plasma effective in completely eliminating both strains at levels of 10^4 after 5 s of exposure and 10^5 and 10^6 after 10 s of exposure. On chicken, plasma exposure resulted in a 1 log reduction following 1 min exposure and up to a 4 log reduction following 10 min of exposure to plasma.

Significance: These data indicate that plasma is effective in reducing *Salmonella* on both agar and chicken surfaces and holds potential as an intervention step in reducing pathogens on the surface of raw poultry.

P2-45 DSC Validation of Ground-and-Formed Beef Jerky Processing Lethality with Commercial Lactic Acid Bacteria Starter Cultures

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Introduction: Illness outbreaks linked to beef jerky have led to increased scrutiny of jerky-making process lethality against *Salmonella* serovars and *Escherichia coli* O157:H7. Ideally, processors should validate their processes with pathogen inoculation studies, but this is impractical. Recent work with whole-muscle beef jerky indicated potential for using commercial lactic acid bacteria starter cultures (LABs) as surrogates for pathogens during in-plant process validation studies.

Purpose: The objective of this study was to identify a commercial LAB pathogen surrogate for evaluating the lethality of ground-and-formed beef jerky processes.

Methods: Initial trials identified two *Pediococcus* spp. LAB cultures as consistently more heat-tolerant than separate five-strain mixtures of *Salmonella* serovars and *E. coli* O157:H7. In five different processes, the thermotolerance of these two LAB cultures was compared to that of an inoculum containing five strains each of *Salmonella* serovars and *E. coli* O157:H7 in ground-and-formed beef jerky formulated with two different spice mixtures. To recover injured cells for enumeration, an overlay plating method used XLD agar for counting pathogen cells and MRS agar for counting LABs.

Results: The two selected LAB cultures were compared individually to each pathogen, resulting in four pathogen/LAB combinations: (1) LAB1 vs. *E. coli* O157:H7, (2) LAB1 vs. *Salmonella*, (3) LAB2 vs. *E. coli* O157:H7, and (4) LAB2 vs. *Salmonella*. For each pathogen/LAB combination, LAB death of > 4.0 log CFU was associated with > 5.0 log CFU pathogen reduction in: 1) 100%, 2) 96%, 3) 94% and 4) 87% of the samples, respectively (n = 26, 26, 30, and 31). Across all combinations, cases where pathogen reduction was < 5.0 log CFU and LAB reduction was > 4.0 log CFU occurred in only 3% of samples (n = 203).

Significance: Our results show that processors of ground-and-formed beef jerky can use commercial LABs as surrogates for *Salmonella* serovars and *E. coli* O157:H7 in validating the lethality of their processes.

P2-46 Validation of Intervention Strategies to Control *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 in Injected Beef at the Retail Level

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Introduction: Recent outbreaks have been linked to the consumption of non-intact meat products contaminated with *Escherichia coli* O157:H7. Purveyors including retailers, supermarkets and restaurateurs rely on meatpackers to control microbial hazards; however, no steps are taken to address this issue other than keeping their products under refrigerated conditions.

Purpose: The purpose of this study was to determine the effectiveness of lactic acid producing bacteria (LAB; ~10^7 CFU/g), acidified sodium chlorite (ASC; 1000 ppm), and lactic acid (LA; 3%) as potential intervention strategies at the retail level to control *E. coli* O157:H7 and *Salmonella* in enhanced beef.

Methods: For each microorganism, USDA choice strip loins were inoculated and refrigerated for 14 or 21 days. After aging, the subprimals were treated with one of the intervention methods, followed by enhancement. Surface and internal counts were enumerated by plating on an overlay of MacConkey Agar and Tryptic Soy Agar (*E. coli* O157:H7) and xylose lysine decarboxylase and Tryptic Soy Agar (*Salmonella*) and incubated overnight at 37°C.

Results: No differences among treatments were observed in *E. coli* O157:H7 surface counts. Internal *E. coli* O157:H7 counts were significantly ($P < 0.05$) reduced by 1.2 logs by application of LAB at day 14 and by > 2.0 logs with ASC at day 21. On day 14 *Salmonella* surface counts were reduced by 0.7 logs after application of LA ($P < 0.05$). Internal *Salmonella* counts were significantly reduced by all interventions, with LAB and ASC presenting the greatest reduction on day 14 (3.0 logs) and 21 (2.5 logs), respectively.

Significance: This study validates the application of LAB, ASC, and LA sprays as effective interventions to reduce translocated pathogens in beef subprimals subjected to injection/enhancement at the retail level. Retailers can use these interventions as an additional measure to improve the safety of their products.

P2-47 DSC Effect of Substrate on Attachment and Subsequent Fate of *Escherichia coli* O157:H7 on Meat-Contact Surfaces

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Introduction: Pathogens attached to meat-contact surfaces may contaminate beef products during fabrication processes and pose subsequent risk to human health.

Purpose: This study evaluated the effect of substrate on: (i) attachment/transfer of *Escherichia coli* O157:H7 onto stainless steel (SS), acetal (AC), polypropylene (PP), and high-density polyethylene (HDPE); and (ii) post-inoculation fate of the pathogen on SS and AC during extended storage.

Methods: Disks (2 × 5 cm) of each material were exposed (30 min) to Tryptic Soy Broth (TSB, pH 7.29), beef fat/lean homogenate (FLH, pH 5.66), fresh conveyor belt-runoff fluid (CBRF, dilute peroxyacetic/octanoic acid, pH 4.60), ground beef (PH 5.76) or beef fat (pH 6.00) inoculated with a 6-strain rifampicin-resistant *E. coli* O157:H7 composite (6 log CFU/ml, g or cm^2). Disks (SS, AC) were then inoculated using the most superior inoculation substrate and incubated (10 days, 15°C) in TSB, FLH or CBRF. Attached cells were recovered from disks by vortexing (3200 rpm, 2 min) and plating on Tryptic Soy Agar with/without rifampicin (two replications, three samples/treatment).

Results: Fat was the most superior surface inoculation substrate ($P < 0.05$), followed by ground beef, FLH and TSB (attachment/transfer of 4.0, 2.5, 2.0 and 1.5 log CFU/cm^2, respectively). No cells were recovered from CBRF immediately after inoculation or from CBRF-inoculated disks. After 3 days (15°C), initial populations (4.0 log CFU/cm^2) on fat-inoculated disks increased by 1.5 and 3.0 log CFU/cm^2 in FLH and TSB, respectively, or decreased by 2.0 log CFU/cm^2 in CBRF, and remained constant throughout storage.

Significance: While culture broth was more conducive to subsequent pathogen growth, fat optimized initial cell attachment/transfer on beef fabrication sur-

faces. This finding may be of significance since *E. coli* O157:H7, if present, should occur on fatty carcass surfaces. However, since decontamination fluids (CBRF) decreased initial attachment and subsequent pathogen levels, their application should be useful in pathogen control at the fabrication level.

P2-48 Effect of Temperature, Shear and Substrate on Attachment and Biofilm Formation by *Escherichia coli* O157:H7 on Various Food-Contact Surfaces Encountered in Beef Processing

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Introduction: Escherichia coli O157:H7 attached to beef-contact surfaces found in fabrication facilities may serve as a source of cross contamination.

Purpose: This study evaluated *E. coli* O157:H7 attachment, survival and growth on food-contact surfaces under beef processing conditions.

Methods: Stainless steel (SS) and high-density polyethylene surfaces (HDPE) (2 × 5 cm) were inoculated with *E. coli* O157:H7 (rifampicin-resistant, six-strain composite; 6 log CFU/ml or g) suspended in Tryptic Soy Broth (TSB; pH 7.28), fat-lean (1:1) homogenate (FLH; pH 5.64) and ground beef (pH 5.75) and incubated (7 days) statically at 4 or 15°C. The effect of incubation conditions was evaluated with SS, acetal, HDPE and polypropylene surfaces contaminated with inoculated beef fat surfaces (pH 6.38, 6 log CFU/cm^2) and stored (15°C, 10 days) statically or under agitation (500 rpm) in FLH (pH 5.70), or statically in empty sterile tubes. Cells on coupons were estimated after vortexing (3200 rpm, 2 min) by plating on Tryptic Soy Agar with/without rifampicin (two replications, three samples/treatment).

Results: Initial attachment of *E. coli* O157:H7 (0.92–2.90 log CFU/cm^2) on SS and HDPE was not affected by temperature but was greater ($P < 0.05$) in ground beef. Adherent and suspended *E. coli* O157:H7 counts increased at 15°C (7 days) by 2.23–5.37 log CFU/cm^2 and 1.04–2.83 log CFU/ml or g, respectively. At 4°C (7 days), although pathogen levels decreased slightly in the substrates, numbers of adherent cells remained constant in ground beef (2.44–2.50 log CFU/cm^2) and increased in TSB and FLH by 0.87–1.04 and 1.74–2.05 log CFU/cm^2, respectively, indicating further attachment. Static/agitation conditions did not affect adherent (4.18–4.83 log CFU/cm^2) and suspended (4.25–4.91 log CFU/ml) counts. Dry storage of surfaces did not reduce counts.

Significance: The results of this study indicate that *E. coli* O157:H7 can attach to beef-contact surfaces not only during fabrication (15°C) but also during cold storage (4°C), rendering the design of more effective sanitation programs necessary.

P2-49 Inactivation of *Escherichia coli* O157:H7 on Raw and Frozen Ground Beef by High Pressure Processing

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Introduction: Ground beef has been implicated in *Escherichia coli* O157:H7 outbreaks because of the possibility of cross contamination during processing. In fall of 2007, the USDA recalled 21.7 million pounds of frozen ground beef patties in a multi-state outbreak of *E. coli* O157:H7. High hydrostatic pressure (HHP), a novel processing technology, has been shown to inactivate pathogens in foods and to preserve texture, color, and nutritional value.

Purpose: The purpose of this study was to evaluate the efficacy of HPP for the inactivation of *E. coli* O157:H7 in ground beef at ambient and low temperatures.

Methods: Ground beef (85% lean) was inoculated with a cocktail of 5 *E. coli* O157:H7 strains at 3 × 10^6 CFU/ml and vacuum packaged. Half of the samples were frozen at -18°C prior to HPP. Inoculated samples of raw beef, frozen beef and Tris-HCl buffer were processed at 20°C or -5°C for 5 or 10 min at 300 or 400 MPa. *E. coli* O157:H7 was enumerated post-processing on Tryptic Soy Agar and selective Sorbitol MacConkey Agar.

Results: Greatest inactivation was found in frozen and buffer samples treated at 400 MPa for 10 min at -5°C and 20°C, with log reductions ranging from 2.7 ± 0.5 to 3.04. Levels of inactivation in raw beef were ~2.1 ± 0.5 for all treatments. Sub-lethal injury assessed by plating on selective media was minimal at 300 and 400 MPa for 5 min but was high for 400 MPa for 10 min at both -5°C and 20°C. Ground beef did not impart an obvious baroprotective effect compared to buffer; however, cells sustained more sub-lethal injury when treated in buffer than in ground beef.

Significance: HPP of packaged ground beef may have potential in the meat industry for the post-process control of pathogens such as *E. coli* O157:H7 at both ambient and low temperatures.

P2-50 DSC Antilisterial Activities of Salad Dressings, without or with Prior Microwave Oven Heating, on Frankfurters during Simulated Home Storage

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Introduction: Consumers show a preference for the use of natural antimicrobials to control foodborne pathogens. Olive oil, lemon juice and vinegar have been reported to exhibit antimicrobial activities in mayonnaise and on vegetables. However, there are no published investigations verifying their antimicrobial activity against *Listeria monocytogenes* on Ready-to-Eat meat products.

Purpose: This study evaluated the antilisterial effects of salad dressings, oil and lemon juice or vinegar, without or with prior microwave oven heating, on frankfurters during simulated home storage.

Methods: Frankfurters (two replicates/three samples each) were inoculated (2.4 ± 0.1 log CFU/cm^2) with *L. monocytogenes* (10-strain mixture) and stored aerobically in bags at 7°C. At 0, 7 and 14 days, frankfurters were dipped (5 or 20 min, 25 ± 2°C) in sunflower oil + lemon juice or vinegar, extra virgin olive oil and lemon juice or vinegar, salad dressings (i.e.,Vinaigrette, Ranch, Thousand Island or Caesar) or distilled water (DW), without or with prior microwave oven heating (1100 Watts, 2450 MHz, high power, 30 s). Samples

were analyzed for microbial growth/survival on Tryptic Soy Agar plus 0.6% yeast extract and PALCAM agar. Data were analyzed using the mixed procedure of SAS.

Results: Dipping in salad dressings and in the combination of oil and lemon juice or vinegar caused significant ($P < 0.05$) reductions of *L. monocytogenes* that were greater than reductions caused by dipping in DW. Reductions increased with product storage, from 0.5–0.9 (day-0) to 1.2–2.1 (day-14) log CFU/cm^2, as levels of contamination also increased. More ($P < 0.05$) reduction of the pathogen was observed when each treatment was applied following exposure to microwave oven heating; reductions ranged from 1.2–1.9 (day-0) to 2.2–3.3 (day-14) log CFU/cm^2. Reductions were not ($P > 0.05$) different between 5 and 20 min of dipping in most treatments.

Significance: Salad dressings, and oil with lemon juice or vinegar, may potentially contribute to control of *L. monocytogenes* on Ready-to-Eat meat products in the home environment.

P2-51 Viability of *Listeria monocytogenes* in Artificially Inoculated Turkey Breast Roll Treated with Lauric Arginate and High Hydrostatic Pressure and Stored at 4°C

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Introduction: Hurdle technology is an effective strategy for control of foodborne pathogens.

Purpose: This study evaluated the effectiveness of lauric arginate (LAE) and high hydrostatic pressure (HHP) for controlling *Listeria monocytogenes* (*Lm*) in turkey breast roll (TBR).

Methods: Tryptic Soy Broth (TSB) and TBR with or without LAE (0 to 1900 ppm) were inoculated with *Lm* to give ~5.0 log CFU/ml and 7.0 log CFU/sample, respectively. A microtiter well dilution assay was used to determine the minimum inhibitory concentration (MIC) of LAE for *Lm* in TSB (37°C). TBR samples (vacuum-sealed) were pressurized (0, 400, 500, or 600 Mpa) at 23°C for 3.0 min and then refrigerated (4°C) for 56 days. At set intervals during storage, TBR homogenates were serially diluted and plated on Modified oxford agar (MOX). *Lm* survivors were determined by counting colonies on MOX after 48 h of incubation (35°C).

Results: The MIC of *Lm* in TSB was 4.94 ppm. Initial numbers of *Lm* in TBR (0 LAE, 0 Mpa) remained unchanged throughout storage. LAE (1900 ppm) reduced initial numbers in TBR by ~0.5 log. Exposure of TBR (without LAE) to 400 and 500 Mpa reduced initial numbers by 4.28 and 6.05 log, respectively. Log reductions in TBR (600 Mpa) were not significantly different from those achieved by 500 Mpa ($P > 0.05$). During storage *Lm* survivors in pressurized TBR (0 LAE) declined gradually. At 56 days numbers of *Lm* in TBR (400 Mpa) were 1.39 log CFU/sample, whereas the pathogen was undetectable following enrichment of TBR (500 or 600 Mpa). Pressurization (600 Mpa) of TBR (containing 1900 ppm LAE) resulted in the greatest decline in viability of *Lm*; numbers of *Lm* in TBR (500 or 600 Mpa) were undetectable beginning at 7 days.

Significance: The use of LAE (1900 ppm) and HHP (500 or 600 Mpa) seems to have good potential for controlling *Lm* in TBR.

P2-52 Controlling *Listeria monocytogenes* in Ready-to-Eat Cooked Meats with Lactate or High Pressure Processing

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Introduction: Listeria monocytogenes is of great concern to the Ready-to-Eat (RTE) meat industry because of its ability to grow at low temperatures. Food safety in these products is normally assured by the addition of lactates and/or diacetates or by the use of High Pressure Processing (HPP).

Purpose: The purpose of this study was to evaluate and compare ingredient (lactate) and process (HPP) approaches to controlling *L. monocytogenes* in RTE meats and to determine if any synergies exist between the approaches.

Methods: Ready-to-Eat ham and turkey samples (with and without added lactate) were inoculated with a cocktail of pressure resistant *L. monocytogenes* strains at a level > 2 $\log_{10}$ CFU/g. Samples were subjected to a 5 min pressure treatment at 600 MPa and 20°C. Corresponding control samples were similarly inoculated but not exposed to high pressure. Samples were stored at 4°C for over 12 weeks and *Listeria* counts were assessed at 2-week intervals throughout storage.

Results: In non-pressure-treated controls, *Listeria* levels increased by ~ 4 $\log_{10}$ CFU/g in 4 weeks at 4°C without lactate whereas growth was significantly inhibited in samples containing 1.9% sodium lactate. *Listeria* levels in samples containing lactate showed ≤ 1 $\log_{10}$ CFU/g increase at the end of 6 weeks and never reached a 4 log increase. Pressure treated samples with or without lactate both showed a decline to below the detection threshold on processing and remained below detection throughout shelf life.

Significance: The data confirm that high pressure processing at 600 MPa for 5 min is an effective post-lethality treatment for control of *L. monocytogenes* in RTE meats. There was no evidence that lactate enhanced HPP effectiveness, although the ability of lactate to reduce the growth rate of *Listeria* may provide incremental protection against *Listeria* growth post-opening.

P2-53 DSC Detection and Identification of *Listeria* spp. at Different Processing Stages of Ready-to-Eat Meat Products Sold in Trinidad

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Introduction: Listeria is one of the most important pathogens found in food, and infections via this organism accounts for 1500 deaths in the US each year. In Trinidad there was a recall by one processing plant due to the contamination of Ready-to-Eat meat products caused by *Listeria*. This may have been a result of poor plant design as well as improper sanitary practices.

Purpose: The aim of this study was to detect and identify routes of product contamination by *Listeria*, including equipment surfaces, raw material and air at all processing stages of chicken franks, chicken bologna and bacon at a processing plant.

Methods: For each product, samples were aseptically collected along the production line from raw materials to the final packaged product. Using standard methods, each sample was enriched in *Listeria* Enrichment Broth for 48 h at 30°C followed by plating onto *Listeria* Selective Agar, and further incubated for 24 h at 37°C. Isolates were confirmed as *Listeria* spp. after subjection to biochemical and slide agglutination tests.

Results: The prevalence was 14.1% for *Listeria* spp., and 2.4% for *Listeria monocytogenes*. The in-process raw meat and ingredients during manufacture of bologna and bacon were positive for the organism at a prevalence of 5.9% each. Although all air and water samples were negative for *Listeria*, the pre-cooking environment of bologna harboured this organism at a prevalence of 0.6% and for bacon, samples were positive for *Listeria* at a prevalence of 1.2% in pre-cooking and 0.6% in post-cooking environments.

Significance: These data suggest that the presence of *Listeria* in the pre-processing factory environment as well as the in-process raw material could be the source of contamination of these products. These results are of immense public health importance and plant processing procedures, together with sanitary practices, need to be immediately addressed.

P2-54 Growth and Survival of *Listeria monocytogenes* in German Sausage

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Introduction: Listeria monocytogenes, the causative agent of listeriosis, is a hardy foodborne pathogen because of its ability to grow at a wide temperature range. Cases of listeriosis have been associated with hot dogs, deli/luncheon meats, Mexican-style soft cheese, butter, raw vegetables, and pasteurized milk.

Purpose: The purpose of this study is to investigate the survival of *L. monocytogenes* at storage and abuse temperatures and to investigate the correlation of the native microflora on the growth and survival of the pathogen.

Methods: The growth and survival of *L. monocytogenes* was evaluated in irradiated versus non-irradiated Teewurst sausage at storage and abuse temperatures. Meat patties were challenged with a five strain cocktail of the pathogen at 10^5 CFU/ml and evaluated on selective and non-selective media. The duration of the experiments was 25 days for 4°C and 15 days for 10°C.

Results: Results from trials I and II in irradiated sausages, from which the native microflora was absent, demonstrated accelerated growth of *L. monocytogenes* at 10°C, from 10^7 to 10^9 colony forming units per gram (CFU/g) at day nine, as compared to 10^4 to 10^6 CFU/g at day fifteen at 4°C. In non-irradiated sausage, *L. monocytogenes* was detected, but at lower levels (10^3 to 10^5 CFU/g). Lactic acid bacteria were the predominant organisms present, with levels reaching > 10^8 CFU/g.

Significance: These results indicate that the native microflora plays a direct role in suppressing the growth of *L. monocytogenes*. The suppression of *L. monocytogenes* may have been the result of the production of bacteriocins by lactic acid bacteria. Understanding of the influences of the native microflora on the survival of *L. monocytogenes* may provide insight to methods leading to pathogen control and disease prevention.

P2-55 Effects of Sodium Lactate, Sodium Citrate, and Sodium Diacetate on Microbiological Quality and Inhibition of *Listeria monocytogenes* in Ready-to-Eat Hams

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Introduction: Listeria monocytogenes is a challenge to Ready-to-Eat meat and poultry processors, as this pathogen may contaminate meat products after the lethality step of production. USDA-FSIS now mandates processors use one of three alternatives to control potential contamination by *L. monocytogenes*. The use of organic acid salts as antimicrobial agents may be used as a control; however, research evaluating the effects of citrate salts at levels exceeding regulatory limits is limited.

Purpose: This study evaluated the use of sodium lactate plus sodium diacetate (SL+SDA); buffered sodium citrate (SC); and SC plus sodium diacetate (SC+SDA) in whole muscle hams to extend shelf life and inhibit the growth of *L. monocytogenes*.

Methods: Whole muscle hams were cured with brine solutions containing one of the following organic acid salts: 0% Control; 2.5% or 3.5% sodium lactate plus sodium diacetate (SL+SDA); 1.3%, 2.5%, or 3.5% buffered sodium citrate (SC); 1.3%, 2.5%, or 3.5% buffered sodium citrate plus sodium diacetate (SC+SDA).

Results: All treatments significantly decreased ($P < 0.05$) ham moisture, while SL+SDA treatments decreased a_w, and hams containing SC decreased smokehouse yields when compared to controls with no organic acid salt. In hams, SL+SDA more effectively decreased aerobic and lactic acid bacteria than formulations with SC or SC+SDA. At both 4 and 10°C, the use of SL+SDA or SC+SDA inhibited aerobic growth longer and increased the inhibitory effects on *L. monocytogenes*. Additionally, the use of buffered sodium citrate (with sodium diacetate) above regulatory limits produced extended shelf life and limited outgrowth of *L. monocytogenes*.

Significance: The addition of sodium lactate or buffered sodium citrate, in combination with sodium diacetate in RTE hams, effectively decreases microbial growth and restricts the growth of *L. monocytogenes* when used at levels higher than 1.3%.

P2-56 DSC Effect of Fat Content on Survival of *Listeria monocytogenes* during Simulated Digestion of Inoculated Beef Frankfurters Stored at 7°C

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Introduction: Little is known concerning the effects of food matrix characteristics on *Listeria monocytogenes* survival during the gastrointestinal stage of infection.

Purpose: This study examined effects of fat content of frankfurters on the ability of this pathogen to survive transit through a simulated gastrointestinal tract.

Methods: L. monocytogenes counts were determined (PALCAM; two replications/three samples each) during storage (7°C, 55 days) of inoculated (2.0–3.0 log CFU/g) and vacuum-packaged frankfurters of low (4.5%) or high (32.5%) fat content, exposed to a dynamic gastrointestinal model (37°C). Controlled parameters included gastric emptying and gastrointestinal fluid secretion rates, gradual gastric acidification (pH reduction to 2.0 within 88 min) and intestinal pH maintenance (6.5 ± 0.3). Survival curves in each gastrointestinal compartment (gastric, GC; intestinal, IC) were fitted with the Baranyi and Roberts mathematical model.

Results: L. monocytogenes populations on low- and high-fat frankfurters exceeded 8.0 log CFU/g at 39 and 55 days of storage, respectively. Major reductions of populations in the GC occurred after 60 min of exposure (pH < 3) of low-fat frankfurters, with reductions at 120 min ranging from 2.6 to > 7.2 log CFU/g at day-1 and day-39, respectively. Corresponding reductions for high-fat frankfurters were 1.6 (day-1) to 5.2 (day-55) log CFU/g. Gastric inactivation rates were 0.080 – 0.194 and 0.030 – 0.097 log CFU/g/min for low- and high-fat samples, respectively. Delivery of gastric contents to the IC began while the gastric pH was still high (> 5). Consequently, counts of contents reaching the IC 30 min after digestion depended on initial product contamination levels. Thus, storage duration affected *L. monocytogenes* cell numbers in the IC, particularly since reductions in populations during the 240-min intestinal challenge were relatively small (0.1–1.4 log CFU/g).

Significance: Findings indicated protective effects of fat against gastric destruction of *L. monocytogenes*. However, effects of fat were observed mainly at later stages of gastric exposure and thus did not influence numbers of viable cells reaching the IC.

P2-57 Effectiveness of Bacteriophage to Control the Outgrowth of *Listeria monocytogenes* on the Surface of Frankfurters

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Introduction: There have been numerous studies published on the effectiveness of surface-applied food grade chemical antimicrobials to control *Listeria monocytogenes* on RTE meat products; however, there is little to no information on the effectiveness of using bacteriophage to control this pathogen in these types of food products.

Purpose: To determine if a commercial, listeriae-specific bacteriophage preparation is effective at controlling *L. monocytogenes* on frankfurters during storage at 4° and 10°C.

Methods: One pound packages of frankfurters were surface inoculated with approximately 3.0 $\log_{10}$ CFU/package of a multi-strain mixture of *L. monocytogenes* and then surface treated with 4 ml of either a 9.0 or 7.0 $\log_{10}$ suspension of bacteriophage. Packages were vacuum-sealed and then placed at 4°C or 10°C for up to 21 days.

Results: Regardless of storage temperature, relative to control packages, the addition of 9.0 $\log_{10}$ of bacteriophage resulted in an initial reduction of about 0.8 $\log_{10}$ of *L. monocytogenes*, whereas there was no appreciable reduction in pathogen levels with the addition of 7 $\log_{10}$. After 21 days at 4°C, pathogen levels increased by about 0.4 $\log_{10}$ CFU/package in control packages, but were about 1.1 and 0.7 $\log_{10}$ CFU/package lower in packages treated with 9.0 and 7.0 $\log_{10}$ of the bacteriophage, respectively. In contrast, at 10°C, pathogen levels increased by at least 5.3 $\log_{10}$ CFU/package in both control packages and packages treated with 9.0 and 7.0 $\log_{10}$ of the bacteriophage preparation after 21 days of storage.

Significance: A listeriae-specific phage preparation can reduce the levels of *L. monocytogenes* on frankfurters by about 1.0 $\log_{10}$ CFU/package. Further studies are warranted to optimize and validate this bacteriophage preparation as a post-processing intervention to control *L. monocytogenes* in RTE meat and poultry products.

P2-58 Cytotoxicity and Genotypic Characterization of *Campylobacter jejuni* Isolated from Poultry Products

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Introduction: Campylobacter jejuni has emerged as a leading cause of gastroenteritis and foodborne illnesses in the United States, due to the consumption of undercooked poultry. *C. jejuni* is considered to be one of the most important foodborne human pathogens of animal origin, with poultry generally considered to be the major reservoir.

Purpose: This study investigated the pathogenicity of *Campylobacter jejuni* strains isolates on Chinese Hamster Ovarian tissue culture cell lines.

Methods: Thirty-one poultry samples were analyzed in this study. The isolates were characterized for their production of catalase and of oxidase, cytotoxic potential with a modified CHO cell assay, 16S rRNA and flagellin (Fla) gene with PCR. In the modified CHO assay, indigenous lactate dehydrogenase (LDH) release was used as an indicator for cytotoxicity.

Results: Ten poultry samples (32%) were positive for *Campylobacter* spp. A total of 50 isolates were obtained, of which 22 (44%) were catalase negative and oxidase positive. Of the 22 presumptive positive *Campylobacter* isolates, 5 (23%) gave positive PCR for both 16sRNA and flagellin (Fla) genes. The release of lactate dehydrogenase from Chinese Hamster cells resulted in cytotoxicity percentage of 99% for *C. jejuni* ATCC 29528, compared to poultry isolates, which exhibited a lactate dehydrogenase release ranging from 33% to 28%.

Significance: These results indicate that the epidemiology of *Campylobacter jejuni* strains isolated from poultry products is of major concern and that the use of a PCR assay applied in combination with biochemical and tissue culture methods is an effective method for isolating *C. jejuni*.

P2-59 Effects of Heat Treatment and Freezing Stress on Survival of *Arcobacter butzleri* Isolated from Chicken

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Introduction: Since *Arcobacter* species were first identified as one of the aerotolerant *Campylobacter* species, *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been known as emerging foodborne pathogens causing food poisoning. These organisms, isolated in chicken, pork, and environmental samples, were reported worldwide.

Purpose: The aim of this study was to investigate the effect of physical stress on growth of *A. butzleri*.

Methods: A. butzleri CAU 080011 and CAU 080024 were isolated with *Arcobacter* selective media from chicken in Korea and submitted to this experiment. Two Korean isolates were treated for 1 min by 40°C,

50°C, 60°C, 70°C, and 80°C heat stress. Survival rate of *A. butzleri* isolates kept under cold (4°C) and freezing (-20°C, and -70°C) conditions was also investigated at 0, 1, 2, 3, 5, and 7 days of storage.

Results: Two and 4 log reductions of *Arcobacter* isolates were found in the 40°C and 70°C heat treatment groups, respectively. No bacterial colony was found in the 80°C group. The number of *Arcobacter* isolates was reduced from 2 log to 4 log in a time dependent fashion regardless of the cold or freezing condition.

Significance: Heating or freezing treatment may be effective methods to control *A. butzleri* contamination of food sources.

P2-60 Growth of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium during the Fermentation of Korean Kim-Chi Supplemented with Pork Meat

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Introduction: Kim-chi is a traditional Korean fermented food made of Asian cabbage, radish, cucumber, and other vegetables with spices including garlic, red pepper powder, etc. In some regions, raw pork, salt water fish, or shellfish are used as the additional ingredients to give better flavor and taste.

Purpose: We investigated the growth pattern of pathogenic bacteria in the pork-supplemented Kim-chi artificially contaminated with *Escherichia coli* O157:H7 and *Salmonella* Typhimurium during the fermentation process.

Methods: Cabbage, radish, and cucumber Kim-chi supplemented with 10% (w/w) pork meat was prepared following a standard recipe and inoculated with 1.0×10^4/g or 1.0×10^6/g concentration of *E. coli* O157:H7 or *S.* Typhimurium ATCC 43174. Each group was fermented at 4°C for 4 weeks and 2 ml of Kim-chi juice was sampled for microbial counts and pH at 1, 2, 3, 5, 7, 10, 15, 21, and 28 days of incubation.

Results: A 3 to 4 log reduction of *E. coli* O157:H7 and *S.* Typhimurium was observed in cabbage and cucumber Kim-chi at 15 days of incubation. *S.*Typhimurium and *E. coli* O157:H7 were not found in radish Kim-chi after 15 and 21 days, respectively. This inhibitory effect on the growth of pathogenic bacteria was statistically significant. Organic acid produced by lactic acid bacteria could be the factor that inhibits the growth of pathogenic bacteria, since the change of pH partially coincided with the growth curve of *E. coli* or *Salmonella* contaminant in Kim-chi.

Significance: Fermentation process of Kim-chi by lactic acid bacteria may reduce the risk of foodborne bacteria contaminated artificially or accidentally.

P2-61 DSC Processing Conditions Associated with *Salmonella* Contamination of Pork Carcasses in Very Small Wisconsin Slaughter Plants

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Introduction: The United States Department of Agriculture (USDA) has expressed concern over the prevalence of *Salmonella* on pork carcasses but does not yet require a validated anti-pathogen intervention treatment.

Purpose: The objectives of this study were to conduct a survey to determine the prevalence of *Salmonella* on pork carcasses in very small Wisconsin plants and to identify processing conditions and indicator bacteria levels associated with reduced *Salmonella* prevalence.

Methods: During April through July, 2007 sponge-samples were obtained from 181 pork carcasses at 10 Wisconsin plants before carcass washing (carcass-half A) and after chilling and before fabrication (carcass-half B). Each sample was categorized by the order in which the animal was slaughtered (1st, 2nd, etc.), whether the carcass was skinned, and wash-water temperature (7°C–43°C), as well as the duration (1 or 2 days), temperature and % relative humidity of chilling. Sponge-samples were qualitatively analyzed for *Salmonella* and quantitatively analyzed for *Escherichia coli*, coliforms, *Enterobacteriaceae*, and Aerobic Plate Count (APC), using Petrifilms.

Results: Salmonella prevalence on carcasses before washing was 12% and 8% for skinned and unskinned carcasses, respectively. Corresponding values for chilled carcasses were 32% and 20% for 1-day chilled carcasses, and 11% and 15% for 2-day chilled carcasses. Lower *Salmonella* prevalence on pre-wash carcasses was significantly related to lower pre-wash carcass APC levels ($P < 0.10$), while lower *Salmonella* prevalence on chilled carcasses was significantly related to a 2-day chilling period, and chilled-carcass coliform, *Enterobacteriaceae*, and APC levels ($P < 0.10$).

Significance: Salmonella prevalence on chilled pork carcasses in very small Wisconsin plants could be reduced by not skinning carcasses and by chilling carcasses two days before fabrication. The trend of greater *Salmonella* prevalence on chilled carcasses vs. pre-wash carcasses also suggests the importance of process hygiene in minimizing *Salmonella* contamination.

P2-62 Serotypes, Intimin Variants and Other Virulence Factors of *eae*-Positive *Escherichia coli* Isolated from Pigs and Sheep at Slaughter

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Introduction: Attaching and effacing *E. coli* (AEEC) are characterized by their ability to cause A/E lesions on intestinal cells. The genes required are located on the LEE pathogenicity island, which has been associated with enteropathogenic and Shiga toxin-producing *E. coli* (EPEC, STEC).

Purpose: The aim of this study was to assess the shedding of AEEC in healthy pigs and sheep at slaughter and to further characterize isolated strains.

Methods: Fecal samples from 198 pigs and 279 sheep were enriched in brilliant green bile broth and examined by PCR for *eae*. AEEC were isolated by colony hybridization and further characterized by serotyping, typing of *eae* and tir, and detection of *stx*, astA, bfpA and EAF.

Results: The proportion of samples with *eae*-positive PCR results was 88.9% for pigs and 54.8% for sheep. Of the isolated AECC, 47.9% of the 48 porcine strains and 64.0% of the 25 ovine strains were typeable for O and H antigens. Only five O26:H11 strains (three from pigs and two from sheep) belonged to a known

human EPEC (and STEC) serotype. Isolated AECC comprised 10 *eae* variants. Among them, intimin θ and β1 dominated (40 strains). Nine strains (mainly of serogroup O145) were positive for intimin γ1 and these strains all harbored *tir*-S (STEC-type). All isolated AEEC tested negative for *stx*, *bfpA*, and the EAF plasmid, but seven strains harbored *astA* (STEC-type). All isolated AEEC tested negative for *stx*, bfpA, and the EAF plasmid, but seven strains harbored *astA*.

Significance: AEEC were found in a remarkable prevalence, but none of the isolated strains harbored *stx* and only a minority belonged to human EPEC serotypes. Finished pigs and sheep constitute a reservoir for atypical EPEC, which are probably underestimated in human infection. The identified O26 (and O145) strains may undergo ephemeral interconversions via loss and gain of *Stx*-encoding phages.

P2-63 *Campylobacter* Transmission Routes in Broiler Flocks on Selected Poultry Farms in Switzerland

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Introduction: Campylobacter are recognized as a major cause of acute bacterial foodborne gastroenteritis worldwide. Broilers are often carriers of *C. jejuni.* Horizontal transmission is believed to be the common way for flock colonization, but the epidemiology in broiler production is still not completely understood.

Purpose: The aim of this study was to investigate the prevalence and genetic diversity of *Campylobacter* in broilers and the environment during several rearing periods.

*Methods: B*roiler flocks, other farm animals, and the environment were examined weekly for consecutive rearing periods on 15 poultry farms. Isolation of *Campylobacter* was accomplished by culture after enrichment. Isolates were identified as *C. jejuni* or *C. coli* by PCR. Genotyping was performed by fla-RFLP (DdeI), macrorestriction profiling with PFGE (SmaI), and AFLP analysis.

Results: Of the 5,154 samples, 311 (6%) from 14 farms were *Campylobacter* positive (mainly *C. jejuni*). Positive samples originated from broilers, broiler houses, cattle, pigs, bantams, laying hens, a horse, and a mouse. Feed, litter, flies, and the supply air to the broiler house tested negative. Certain genotypes from cattle, pigs or laying hens were subsequently found in the broiler flocks (seven farms). Otherwise, certain genotypes from the flocks appeared subsequently in cattle or laying hens (four farms). On four farms, matching genotypes were detected in consecutive broiler flocks, but not concurrently in other samples. Some genotypes were identical across different farms.

Significance: The results of this study emphasize the importance of other farm animals as reservoirs for poultry flock colonization and illustrate the role of personnel moving between areas as potential vectors. However, more potential vectors or niches may exist because the described sources could be excluded for some genotypes.

P2-64 Predictive Model for Growth of *Clostridium perfringens* during Cooling of Cooked Ground Chicken

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Introduction: Traditional methodologies for development of microbial growth models under dynamic temperature conditions do not take the organism's history into consideration. Such models were shown to be inadequate in predicting growth of the organisms under dynamic conditions commonly encountered in the food industry. Therefore, incorporating an assumption that growth kinetics depends in an explicit way on the cells' history provides accurate estimates of growth or inactivation.

Purpose: The objective of the current research was to develop a predictive model for *C. perfringens* spore germination and outgrowth in cooked chicken products during coolin, by incorporating a function to describe the prior history of the microbial cell in the secondary model.

Methods: Cooked ground chicken was inoculated with *C. perfringens* spores and vacuum packaged. For the isothermal experiments, all samples were incubated in a constant temperature water bath stabilized at selected temperatures between 10°C and 51°C and sampled periodically. The samples were cooled from 54.4 to 27°C and subsequently from 27 to 4°C at different time periods (cooling rates) for dynamic cooling experiments.

Results: For a temperature decline from 54.4°C to 27°C in 1.5 h, the standard model predicted a $\log_{10}$ relative growth of about 1.15, with a standard error (SE) of about 0.065 $\log_{10}$, while the mean of observed results for two replicates was 0.47 $\log_{10}$. For the same temperature decline in 3 h, the predicted $\log_{10}$ relative growth was about 3.3 $\log_{10}$ (with a SE of about 0.07), and the mean of the observed $\log_{10}$ relative growths were 2.7 $\log_{10}$. However, for a selected memory model, an estimate of $\log_{10}$ relative growth for the above cooling scenario was 0.76 $\log_{10}$, within 0.3 $\log_{10}$ of the observed mean. For other cooling scenarios this memory model provided predictions within 0.3 $\log_{10}$ of the mean observed $\log_{10}$ growth values.

Significance: These findings point to an improvement of predictions obtained by memory models over those obtained by the standard model. The predictive model will be of immediate use to the retail food service operations and regulatory agencies to aid in the disposition of products subject to cooling deviations and therefore, ensure the safety of the cooked foods.

P2-65 DSC The Survival of *Salmonella* during Frozen Storage and Microwave Cooking of Chicken Products

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Introduction: Frozen chicken products have been identified recently as sources of the cause of salmonellosis. Microwave heating often produces an uneven temperature distribution, and its use for cooking frozen chicken products containing raw poultry is currently a matter of concern.

Purpose: The objectives of this study were: to assess survival of *Salmonella* during frozen storage when inoculated in a chicken matrix, and to evaluate lethality of microwave cooking of frozen chicken products inoculated with *Salmonella* when those products are heated according to the package directions.

Methods: Four *Salmonella* strains originally isolated from poultry were inoculated into frozen chicken nuggets at initial populations of 10^7 CFU/g and 10^4 CFU/g. Survival was assessed weekly during storage at -20°C by measurement of bacterial growth on complete and selective agar. The same 4 *Salmonella* strains were inoculated at 10^6 CFU/g into frozen chicken entrées

Poster

containing raw poultry. Samples were inoculated at 3 different depths, and subjected to heating in a research microwave oven at power settings corresponding to full power for 500, 1000 and 1300 Watt microwave ovens, and cooked according to the label's instructions. Product temperatures in different locations were monitored using fiberoptic thermometry.

Results: Frozen storage results indicate that after an initial decrease of ~1 log CFU during the first week, bacterial levels remain constant during 14 weeks of storage at -20°C. Bacterial counts were lower in selective agar, suggesting that these cells were structurally injured. Microwave cooking results showed that *Salmonella* inoculated in a frozen chicken entrée (at 10^6 CFU/g) that was cooked following the label instructions in a 500W microwave oven was still detected after treatment.

Significance: The data presented in this study indicate that *Salmonella* can survive frozen storage when inoculated in a chicken matrix, and that the use of a lower wattage microwave oven for cooking frozen products containing raw poultry may be an unsafe practice.

P2-66 DSC Validation of a 2% Lactic Acid Antimicrobial Rinse as an Alternative to Chlorine for Mobile Poultry Slaughter Operations

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Introduction: Poultry is a known source of several foodborne pathogens; therefore, processing interventions to reduce pathogens are critical. Washington mobile poultry slaughter operators wanted to identify an antimicrobial rinse alternative to chlorine to assist with product organic labeling and increase appeal to consumers. Lactic acid has proven an effective antimicrobial rinse in red meat processing.

Purpose: The effectiveness of lactic acid and chlorine rinses in mobile poultry slaughter operations was examined; the incidence of *Salmonella* on carcasses was also examined.

Methods: Carcasses were sampled either immediately after evisceration (no rinse control, NR), after a 3 min 50–100 ppm chlorine rinse (CHL) or after a 3 min 2% lactic acid rinse (LA). Two replications were performed; for each replication, twenty carcasses per treatment (NR, CHL, LA) were randomly selected. For microbial sampling, whole carcasses were rinsed with 0.1% peptone water, and rinse fluid was examined for aerobic plate count (APC) on Tryptic Soy Agar (TSA) and total coliforms on Violet Red Bile Agar. NR carcasses were examined for the presence of *Salmonella* by use of standard plating and biochemical methods.

Results: No *Salmonella* were detected on NR carcasses. As expected, CHL (3.78 CFU/ml) and LA (2.26 CFU/ml) APC were significantly ($P < 0.01$) lower than NR (4.28 CFU/ml) APC. The LA (2.26 CFU/ml) APC were significantly ($P < 0.01$) lower than CHL (3.78 CFU/ml) APC. Furthermore, LA total coliforms were significantly ($P < 0.01$) lower than CHL (2.93 CFU/ml) and NR (3.12 CFU/ml) total coliforms. Interestingly, there was no significant ($P = 0.10$) difference between CHL (2.93 CFU/ml) and NR (3.13 CFU/ml) for total coliforms.

Significance: Clearly, LA was more effective than CHL at reducing both total coliforms and APC. The 2% lactic acid rinse not only was validated as an alternative to 50–100 ppm chlorine, it was also shown to provide an increased reduction in APC and total coliforms, making it an attractive option for mobile poultry slaughter operations.

P2-67 DSC Effect of Kosher Salt Application on Microbial Profiles of Poultry Carcasses

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Introduction: Most chemical interventions currently employed by poultry processors have had limited success in consistently reducing bacterial loads. In addition, there are elevated concerns from consumers on the use of chemicals to treat foods; thus, more natural alternatives need to be explored. Kosher salting of poultry has been used for centuries as a process to remove remaining blood from carcasses during processing. However, the antimicrobial effect of salting on chicken carcasses has not been elucidated.

Purpose: The current study was aimed at evaluating the antimicrobial effect of conventionally applied kosher salt on the microbiological profile of chicken carcasses.

Methods: Broiler carcasses obtained post evisceration from a commercial processing facility were divided into five groups: a non-treated control, a chilled control, pre-chill kosher salt application (sampled before and after chilling), and a post-chill kosher salt application (applied to chilled carcasses). Standard sampling methods were used to evaluate *Salmonella* prevalence, aerobic plate counts, coliforms, generic *Escherichia coli* and psychrotrophs.

Results: Results indicate significant reductions in microbial populations in all the groups compared to controls. Significant reductions (1.3, 2.5, 1.3, and 2.7 log CFU/ml of rinse) ($P < 0.05$) were obtained for APCs, coliforms, psychrotrophs and *E. coli* respectively on pre-chill salt treated carcasses when compared to controls. Salt treated carcasses sampled after chilling had lower microbial populations compared to control chilled samples, with significant reductions in coliforms and *E. coli* (0.8 and 1 log respectively) ($P < 0.05$). *Salmonella* was not detected in any of the samples, including controls. Salt treated samples had lower counts on APCs and psychrotrophs after 10 days of refrigerated storage (0.8 and 1.1 log reductions, respectively) when compared to controls ($P < 0.05$).

Significance: Based on the results, it can be concluded that salt is an effective antimicrobial that needs further investigation as a possible intervention in commercial poultry processing settings.

P2-68 Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Retail Meat: National Antimicrobial Resistance Monitoring System (NARMS): 2002–2006

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Introduction: Salmonella spp. is frequently associated with foodborne illness in humans and is commonly isolated from food animals and their derived meats. Multidrug resistant variants have emerged, reducing therapeutic options in cases of invasive infections in both human and animals.

Purpose: The purpose of this study is to investigate the prevalence, antimicrobial susceptibility, and genetic diversity of *Salmonella* recovered from retail meats.

Methods: We compared the prevalence of *Salmonella* in a sampling 20,295 meats, including chicken breast (n=5,075), ground turkey (n=5,044), ground beef (n=5,100) and pork chops (n=5,076) collected during 2002-2006 for the NARMS retail meat surveillance program. Isolates were analyzed for antimicrobial susceptibility and genetic relatedness using PFGE.

Results: From a total of 1,380 *Salmonella* isolates recovered, 668 (48.4%) were from ground turkey, 607 (44%) from chicken breast, 62 (4.5%) from ground beef and 43 (3.1%) from pork chop. The top five serotypes were S. Heidelberg (n=298, 21.6%), Kentucky (n=203, 14.7%), Typhimurium (n=178, 12.9%, including Typhimurium var.Copenhagen and S.I 4,5,12:i:-), Saintpaul (n=110, 8 %), and Hadar (n=91, 6.6%). Eight hundred seventy-three (63.3%) isolates displayed resistance to >1 of the 15 antimicrobials tested, and 191 (13.8%) were resistant to >5 antimicrobials. Twenty-four (1.7%) isolates from 7 different serotypes recovered from all four types of meats exhibited resistance to ≥9 antimicrobials. Resistances frequencies for the tested antimicrobials were tetracycline (44.9%), streptomycin (35.4%), sulfamethoxazole (26.1%), and ampicillin (25.5%), and to lesser extent, to gentamicin (14.9%), amoxicillin-clavulanic acid (14.2%), kanamycin (13.6%), ceftiofur (12.7%), cefoxitin (12.65%), chloramphenicol (3.1%), nalidixic acid (1.6%), trimethprim-suflamethoxazole (0.9%) and ceftriaxone (0.6%). All isolates were susceptible to amikacin and ciprofloxacin. PFGE analysis using XbaI and BlnI indicated a genetically diverse population, however, several clones were repeatedly recovered from different retail meats, brands and stores over the 5 year period.

Significance: Results indicate that multiple antimicrobial-resistant salmonellae are present in retail meats, in particular poultry products, and stress the need for sustained surveillance of foodborne pathogens in retail foods.

P2-69 DSC Thermal Inactivation of *Salmonella* Enteritidis in Egg Constituents by Traditional Processing and in In-Shell Eggs by Microwave Processing

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Introduction: According to the USDA, 1 in 20,000 eggs, or about 2.7 million eggs annually, are infected with *Salmonella* Enteritidis (SE). Microwave pasteurization of shell eggs would make an important contribution to the reduction of the threat of foodborne salmonellosis. To determine if pasteurization is achieved in a particular microwave applicator, biological testing with an appropriate SE strain is required.

Purpose: To characterize the relative thermal resistance of seven SE strains in phosphate buffer saline and study the inactivation of the relatively heat resistant SE strain in egg constituents (i.e., yolk, albumen and liquid whole egg) at various temperatures; to monitor the growth kinetics of the chosen SE strain in intact shell egg at 40°C, a temperature frequently encountered in commercial layer operations, and study the shell egg pasteurization effectiveness of a proprietary microwave applicator to achieve ≥ 5-$\log_{10}$ SE reduction.

Methods: A polystyrene card holder method was developed to accommodate the highly viscous egg constituents. Thermal inactivation studies were carried out with this method, using a water bath. SE inoculated shell eggs were processed by a proprietary microwave applicator.

Results: In buffer, SE H7037 proved to have the highest heat resistance resulting in D-values of 3.51 and 1.75 min at 55 and 57°C, respectively. Thermal inactivation kinetics (D- and z-values) were obtained in albumen (55°C, 56°C, 57°C), egg yolk (58°C, 60°C, 62°C) and liquid whole egg (55°C, 57°C, 60°C). The z-values were found to be 6.12°C (yolk), 4.63°C (albumen) and 5.03°C (liquid whole egg). Experiments with the microwave applicator achieved a 5-$\log_{10}$ SE reduction, but the results were inconsistent.

Significance: Microwave process optimization studies need to be carried out in order to achieve a reproducible, 5-$\log_{10}$ SE inactivation in shell eggs for the purpose of pasteurization.

P2-70 DSC Effect of Ozone Concentration on Inactivation of *Salmonella enterica* Serovar Enteritidis in Shell Eggs by Sequential Application of Heat and Ozone

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Introduction: Shell eggs are the most common vehicle for human infection by *Salmonella enterica* serovar Enteritidis. Many cases of egg-related salmonellosis are reported annually despite efforts to reduce contamination, including thermal pasteurization of shell eggs and egg products.

Purpose: To assess the feasibility of eliminating *Salmonella* Enteritidis in experimentally inoculated whole shell eggs, using heat and ozone combination treatments in pilot-scale equipment.

Methods: Whole shell eggs were inoculated with low levels of *Salmonella* Enteritidis (4.9–5.4 log CFU per egg) near the egg vitelline membrane. Eggs were subjected to immersion heating (57°C for 21 min) or a combination of heating and ozone treatment (67.5 kPa vacuum, followed by ozonation at an approximate maximum concentration of either 14, 28, or 140 g/m^3 ozone in oxygen and 184.1–197.9 kPa for 40 min). Surviving *Salmonella* were enumerated with a modified most-probable number technique.

Results: Heat treatment alone inactivated 3.1 log *S.* Enteritidis per egg, and combination treatments using 14, 28, and 140 g/m^3 ozone produced reductions of 3.0, 2.9, and 4.2 log *Salmonella* Enteritidis per egg, respectively. There was no statistical difference ($P < 0.05$) in the inactivation observed as a result of heat alone or combination treatments utilizing 14 or 28 g/m^3 ozone. Inactivation by treatment with heat and 140 g/m^3 ozone was significantly greater than that resulting from all other treatments.

Significance: Data from this study suggest that a combination treatment of heat and high level ozone has the potential to greatly increase safety of whole shell eggs. Use of ozone may allow a reduced heating time compared to heat pasteurized eggs, possibly resulting in a higher quality end product.

P2-71 Evaluation of Glass Capillary Tube and TDT Disk Methods for Determining Thermal Inactivation Kinetics of *Salmonella* in Liquid Whole Egg

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Introduction: The USDA liquid egg pasteurization standards are based on the thermal inactivation of *Sal-*

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monella. Glass capillary tubes traditionally have been used to determine thermal death times of microorganisms in liquid foods. A new thermal-death-time (TDT) disk is a reportedly simpler means for evaluating bacterial inactivation in liquids, providing up to one milliliter of sample for microbiological analysis.

Purpose: The goal of this comparative study was to determine the thermal inactivation kinetics of *Salmonella* in liquid whole egg (LWE) using the glass capillary tube and TDT disk methods.

Methods: A five-strain-mixture of *Salmonella*, representative of common egg-related serovars, was inoculated at ca. 8 log CFU/ml into LWE, and injected (0.025 ml per tube) into glass capillary tubes, which were flame-sealed. Inoculated LWE (0.9 ml) was also added to TDT disks. Capillary tubes and TDT disks were immersed in a circulating water bath at 54, 56 and 58°C, and temperatures were measured with thermocouples. Two capillary tubes and one TDT disk were removed at each sampling time and immersed in an ice slush bath; samples were plated for enumeration onto Tryptic Soy Agar with 1 g/L sodium pyruvate and 6 g/L yeast extract. Regression analysis was performed, D- and Z-values were calculated, and significant differences between methods were determined by *t*-tests.

Results: The D-values for the capillary tube method were 13.08, 4.49, and 1.19 mins at 54, 56, and 58°C, respectively, and 11.15, 3.41, and 1.11 min., respectively, for the TDT disk method. The Z-values were calculated at 3.99 for the capillary tube method and 3.84 for the TDT disk method. No significant difference ($P > 0.05$) was found between methods.

Significance: The capillary tube and TDT disk methods provide equivalent D and Z- values for the thermal inactivation of *Salmonella* in LWE, although the latter method provides a twenty-fold larger sample volume for microbiological analysis.

P2-72 Occurrence of *Campylobacter* in Commercially Shelled Liquid Egg in Japan

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Introduction: Campylobacter is a foodborne human pathogen known to contaminate poultry products. While contamination levels and numbers of the bacteria in poultry meat are well documented, there are few reports on contamination of commercially shelled liquid egg by *Campylobacter*.

Purpose: We investigated the level of contamination of liquid egg by *Campylobacter*, and also studied if the legal pasteurization condition of liquid egg for *Salmonella* in Japan could be used for *Campylobacter*.

Methods: From 10 egg shelling facilities in Japan, 140 non-pasteurized and 110 pasteurized liquid egg samples were collected. These liquid egg samples were frozen at the facilities and shipped to our laboratory. The number of *Campylobacter* was determined by selective enrichment and the MPN procedure. Their heat tolerance was observed by use of the method with heat treatment at different temperatures.

Results: Out of the 140 non-pasteurized liquid egg samples, 39 samples were positive for *Campylobacter* (27.9%), while no *Campylobacter* was found in the pasteurized liquid egg samples. The contamination levels of all the contaminated samples determined with the MPN procedure were less than 28 microorganisms per 100 ml. It was demonstrated that *Campylobacter* is sensitive to heat, with its D-values of 0.44–1.86 min and 0.12–0.32 min at 55°C and 58°C, respectively.

Significance: This very weak heat tolerance of the bacteria in liquid egg confirmed that there is no safety concern of *Campylobacter* contamination of commercially shelled pasteurized liquid eggs.

P3-01 Innovative Strategies to Enhance Food Safety in the Hospitality Industry in Dubai

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Introduction: The cosmopolitan nature of Dubai is reflected in the different world populations residing here, as well as in its food culture. This small city hosts 124 four and five star hotels, with another 20 new hotels slated to begin functioning in the next year alone. This leap in growth has made obvious the lack of scientific expertise, equipped laboratories, and trained personnel. Added to this is a highly volatile job market that has grown exponentially in the past couple of years.

Purpose: The aim of this project undertaken by the Dubai Municipality was to provide a better understanding of HACCP to the hospitality industry and to enable businesses to adapt this knowledge to help meet the requirements of legislation.

Methods: Online discussions, team meetings, training programs, and field visits were arranged for industry personnel, in conjunction with regulatory officials. These programs aimed to enhance communication between the two groups and enhance performance through team work and interactive programs, as well as to improve understanding on food safety issues, identify problem areas, and provide expertise, ultimately enhancing voluntary control. Dubai Municipality also organizes an international conference every year to bring in experts from around the world to interact with the local workforce with an aim to improve knowledge sharing on important issues related to food safety.

Results: A definite improvement in the food safety culture of the industry was noticed. Almost 50 hotels were HACCP certified by mid-2008. Increased hygiene awareness was noticed, with more food safety officers being recruited, and complete training was provided for all food handlers. Government inspection reports showed reduced violations, in addition to improved communication and co-operation between regulatory authorities and industry.

Significance: Rational and innovative strategies to increase awareness are beginning to show results in improving the food safety standards with regard to the hospitality industry in Dubai.

P3-02 Comparison of Sample Processing Methods for Detection of Staphylococcal Enterotoxin B by an Electrochemiluminescence (ECL) Immuno-Based Assay

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Introduction: Current methods for detection of staphylococcal enterotoxin B (SEB) from foods are limited by the inefficiency of toxin recovery after extraction, particularly when low toxin concentrations are present. Detection protocols including sample preparation, extraction and detection should be able to detect at least 1 ng of SEB/g of food. Food matrix effects are important contributors to assay sensitivity and preparation protocols should be optimized for the specific assay and food matrix being utilized.

Purpose: The purpose of this study was to evaluate the efficiency of three sample processing methods for recovery of low levels of SEB from food products, using a commercial ECL-based detection technology (BioVeris™ M1M).

Methods: Foods samples (diced ham, instant infant formula, liquid whole eggs and Romaine lettuce) were inoculated with SEB (0, 0.1, 0.5 and 1 ng/ml). Samples were homogenized in 0.1 percent peptone buffer in stomacher bags with and without filters, using either a Stomacher 400 or Pulsifier lab blender. Homogenized samples were further prepared utilizing commercial BioVeris SEB kits that include an additional filtration step. SEB detection sensitivity by ECL was determined based upon standard curves and signal-to-background ratios (S:B) of the instrument.

Results: SEB was consistently detected down to 0.5 ng/g or ml in all food matrices except Romaine lettuce. For all food types, the samples processed using the Pulsifier yielded comparable or higher S:B ratios compared to the Stomacher method, even when the secondary BioVeris kit filtration step was eliminated. Similar SEB detection sensitivity was observed for liquid whole eggs in Stomacher bags without filters using either the Pulsifier or Stomacher 400 system. Romaine lettuce extract seemed to interfere with ECL detection of SEB with use of the commercial kit, with negative controls yielding a positive detection signal.

Significance: These data suggest that primary homogenization of food samples with the Pulsifier system provides a cleaner processed sample for SEB detection utilizing the BioVeris M1M ECL-based technology. With the Pulsifier system, the secondary filtration step incorporated into the commercial BioVeris extraction kits was not necessary for the food matrices evaluated.

P3-03 Validation of Sample Compositing for Detection of *Escherichia coli* O157:H7 on Spinach in Conjunction with Traditional and Rapid Methods

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Introduction: Sample compositing is commonly used for analysis of food products, but no universal validation has been performed. Sample sizes as large as 600 g have been suggested for leafy greens but it is currently unknown if existing compositing approaches are applicable.

Purpose: Our objective was to evaluate the effect of compositing on detection of *Escherichia coli* O157:H7 on spinach, using immunological and molecular detection techniques.

Methods: Twenty-five gram portions of spinach were inoculated with a four-strain cocktail of *Escherichia coli* O157:H7 at 0.1 cells/gram. These portions were combined with uninoculated greens just prior to the analysis, to obtain samples of three sizes: 25, 125, and 375 g. The inoculation level was determined with an additional portion of inoculated sample and uninoculated portions were used as negative controls. The inoculated leafy green vegetables were tested for detection of *E. coli* O157:H7, using the standard protocols for testing for BAX MP, SDI, and Biocontrol GDS Assurance methods compared to the FDA BAM cultural method. The portion positive for each of the 3 test methods after 8 and 24 h of incubation were compared to portion positive for the FDA BAM method after 24 h of incubation, using a Chi-square analysis for unpaired samples.

Results: The GDS Assurance method performed as well as the reference method for all sample sizes at 8 hr or 24 h of incubation. The SDI method performed as well as the FDA BAM method with a 25 g sample at 8 h. With 8-h incubation, the BAX MP method performed as well as the reference method for 25 and 125 g samples. At 24 h, both the SDI and BAX methods performed better than the reference method with all sample sizes.

Significance: Sample sizes of up to 375 g are a viable option for the testing of spinach for *E. coli* O157:H7.

P3-04 Evaluation of BD Bacto™ Tryptic Soy Broth and Difco™ EC Medium, Modified for the Qualitative Detection of *Escherichia coli* O157:H7 in Beef Trims when Tested with Commercial Test Kits

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Introduction: Research has reported the successful use of non-proprietary enrichment media when enriching ground beef and beef trimmings for the detection of *Escherichia coli* O157:H7. However, non-proprietary enrichment needs to be assessed when the final detection method is an immunoassay or a molecular detection system.

Purpose: The objective of this study was to evaluate BD Bacto™ Tryptic Soy Broth (TSB) and Difco™ EC Medium (EC), Modified as enrichment media for detection of *E. coli* O157:H7 in beef trims when using: BAX *E. coli* O157:H7, RapidChek™ *E. coli* O157:H7, and the GDS Assurance™ *E. coli* O157:H7 test kits.

Methods: Portions of beef trims were inoculated, at 1–5 cells per 65 g sample for low inoculation level and 10–50 cells per 65 g for high inoculation level, with a two-strain cocktail of *E. coli* O157:H7. Inoculated samples were enriched in a 1:10 dilution of TSB, modified EC, and the proprietary media of each test kit method. The proprietary media were incubated at the prescribed temperature of the individual test kit for 8 and 24 h. The TSB and modified EC broth were incubated at 42 ± 1°C for 8 and 24 h. Each broth was analyzed as prescribed by the individual method, using automated BAX *E. coli* O157:H7 MP Test (DuPont-Qualicon), RapidChek *E. coli* O157:H7 Test (Strategic Diagnostics), and GDS *E. coli* O157:H7 (BioControl). In addition, each sample was struck for isolation onto BBL CHROMagar O157.

Results: For the detection of *E. coli* O157:H7 from inoculated beef trims, Modified EC and TSB performed as well as or better than the proprietary media for BAX, SDI, and GDS test methods at both 8 and 24 h and with low and high inoculum level.

Significance: Non-proprietary media can be used for the detection of *E. coli* O157:H7 when using immunoassays or molecular detection systems.

P3-05 Evaluation of a Molecular Beacon Based Real-Time PCR Test for Detection of *Salmonella* spp. in Selected Foods from a Single Primary Enrichment

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Introduction: Salmonella is one of the most commonly reported causative agents of bacterial foodborne illness in the United States, causing an estimated 1.4 million illnesses per year. There are testing methods available for the detection *Salmonella* in foods but these methods take at least 3 days for a presumptive result. iQ-Check *Salmonella* II is a real-time PCR kit utilizing specific oligonucleotide probes, called molecular beacons, to detect *Salmonella* spp. in food. The test method allows for the detection of a presumptive result from a food sample in less than approximately 24 h, utilizing a single primary enrichment in Buffered Peptone Water.

Purpose: The objective of this study was to compare the performance of the method to that of established USDA and FDA reference methods for chicken, beef trim, whole shell eggs, and cantaloupe.

Methods: Food was screened for indigenous *Salmonella* prior to inoculation. Twenty 25 g low-level inoculated samples and 5 uninoculated control samples of each food were tested for *Salmonella,* using the test method and the appropriate reference method. In addition, a MPN analysis was performed for each food type in order to determine the level of *Salmonella* present in the food samples.

Results: A Chi-square analysis according to McNemar for paired samples and Mantel-Haenszel for unpaired samples revealed no significant difference between the number of samples determined to be positive by the test method and that of the appropriate reference method for chicken, beef trim, whole shell eggs, and cantaloupe. Overall method agreement was 94%. The test method identified more true confirmed positive samples than the reference method.

Significance: The method can be used to detect the presence of *Salmonella* in foods in less time than is required by the traditional reference methods.

P3-06 Comparison of a Novel Sample Collection Device and Buffer with a Cellulose Sponge for the Collection and Detection of *Listeria,* Using the USDA-FSIS Culture Method

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Introduction: Listeria spp. detection and monitoring is important in food processing plant safety management because it can be an indicator of possible *L. monocytogenes* contamination. Current collection methodologies for environmental samples often employ a cellulose sponge hydrated in a neutralizing buffer. The use of a sponge, however, typically requires large sample volumes. In addition, without significant dilution, many neutralizing buffers can be inhibitory to molecular detection methods.

Purpose: We have developed a new assay based on Reverse-Transcriptase PCR for detecting the genus *Listeria* from environmental surfaces. Product requirements included a sampling device that was compatible with small volumes and a collection buffer that was not inhibitory to the test method. Another requirement was that the device/buffer perform similarly to a cellulose sponge, as measured by culture. We therefore developed a novel sample collection device using DuPont nonwoven materials and a non-inhibitory collection buffer. The purpose of this study was to evaluate performance of the DuPont wipe/buffer against a cellulose sponge, using spiked surface samples.

Methods: DuPont collection wipes, hydrated with either the DuPont collection buffer or D/E buffer, were compared to a cellulose sponge hydrated with D/E buffer by use of a version of the USDA-FSIS culture method. A total of 80 stainless steel, plastic and ceramic tile surfaces were inoculated with either *L. welshimeri, L. seeligeri, L. innocua* or *L. ivanovii* at a level previously determined to give fractional positive results.

Results: Mantel-Haenszel Chi-square analysis of the data showed no significant difference ($X^2 = 0.08555$ or 0.00) between the D/E buffer cellulose sponge collection and either of the DuPont wipe/buffer configurations, across all surface types and organisms.

Significance: This data indicates that the DuPont wipe and buffer were equivalent to the cellulose sponge, based on culture performance. Unlike the cellulose sponge, the DuPont wipe and buffer are fully compatible with the downstream detection technology.

P3-07 Effect of Time, Temperature, and Neutralizing Media on the Recovery of Aerobic and Coliform Bacteria from Environmental Sponges

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Introduction: It is generally recommended that environmental sponges be processed within 24 h of collection and stored/shipped under refrigerated temperatures when not analyzed immediately after collection. Because of time delays and/or temperature deviations encountered during shipping of environmental sponges to remote laboratories, it is necessary to understand conditions that adversely affect the results of microbiological analysis.

Purpose: This study evaluated the effect of storage time, temperature, and neutralizing media on the recovery of aerobic and coliform bacteria from environmental sponges.

Methods: Stock cultures of aerobic and coliform bacteria were individually cultivated in BHI broth. Equal volumes of each culture were pooled and used to inoculate 500-ml volumes of Den/Engley Neutrallzing (DE) broth and Neutralizing (N) buffer. Ten ml aliquots of inoculated DE broth or N buffer were then added to dehydrated sponges such that the initial inoculation levels were 4–5 log CFU/sponge. Sponges were stored at -20°C, 4°C or 12°C and five replicates were analyzed at 0, 24, 48, 72 and 96 h for aerobic plate count and total coliforms, using appropriate 3M Petrifilm.

Results: After 24 h storage (all three temperatures) of sponges rehydrated with N buffer, populations of APC and coliforms were significantly lower ($P < 0.05$). The results for sponges rehydrated with DE broth stored at -20°C were the same. When sponges rehydrated with DE broth were stored at 4°C, significantly greater ($P < 0.05$) populations of APC and coliforms were recovered after 48 and 72 h, respectively. After 24 h storage at 12°C, sponges rehydrated with DE broth also had significantly greater ($P < 0.05$) populations of APC and coliforms.

Significance: These data suggest that environmental sponges rehydrated with N buffer must be analyzed for APC and coliforms within 24 h of sampling. DE broth may be a more suitable neutralizing medium for sustaining bacterial viability in sponges.

P3-08 Novel System for the Detection of Indicator Organisms in Swabs and UHT Products

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Introduction: Most indicator organisms such as total aerobic count (TC), yeast and mold (YM), coliform and *E. coli* (*EC*) can be determined by the time consuming plate count methodology. In recent years the application of rapid automated methods as alternatives to the plate count method has become increasingly important. BioLumix has developed a new optical system for rapid automated detection of indicator microorganisms.

Purpose: To evaluate the new system and technology for the detection of TC, YM, coliform and *EC*, and show its applicability to environmental testing and UHT products.

Methods: The new system detects optical changes due to microbial growth in liquid growth medium containing optically sensitive reagents (color or fluorescence). A transparent sensor for the detection of CO_2, was used for TC and YM. The simultaneous detection of coliform and *E. coli* was achieved with a color and fluorescent dyes combination. The surface of stainless steel coupons was inoculated with 83 different strains of bacteria, 14 molds and 12 yeasts. All were detected by the CO_2 sensor, while none of the un-inoculated samples gave positive results. Stainless steel coupons inoculated with various types of bacteria, yeast and mold were swabbed. The inoculated swabs were tested for TC, YM or coliform/EC combination. UHT products were evaluated for the presence/absence of microorganisms, using the BioLumix system.

Results: The coliform/EC vial was capable of distinguishing between coliform and *E. coli* in environmental samples. 37 store bought UHT products, including milk, half and half, shakes, and whipped cream, were tested in the system. An additional 76 products were inoculated with low numbers of bacteria or yeast. While contaminated product was detected by the system, none of the clean samples gave positive results.

Significance: The data suggest that the new method is useful for determining total aerobic bacteria, yeast and mold as well as coliform and *E. coli*.

P3-09 Comparison of Swiffer® Wipes and Conventional Drag Swab Methods in the Recovery of *Salmonella* from Swine Production Environment

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Introduction: Salmonella organisms can survive in the environment for long periods, during which they can be a source of infection for food animals such as pigs, poultry and cattle, which in turn are considered important sources of human infections. In implementing *Salmonella* monitoring programs in food animal production environments, a sampling method of the environment which is convenient to apply in the field, is cost-effective and could result in better recovery of *Salmonella* is required. Various studies have reported the results of different sampling methods for the detection of *Salmonella* in poultry farms. However, there is paucity of information on the methods of sampling for the detection of *Salmonella* in swine production environment.

Purpose: The purpose of this study was to assess the efficacy of Swiffer® wipes as compared to conventional drag swab sampling methods for the recovery of *Salmonella* in swine production environment.

Methods: Swiffer® wipes (n = 352) and drag swab samples (n = 352) were aseptically collected from 15 barns before disinfection (pre-) and between 30 mins and 2 h after completion of disinfection (post-) of each barn and before placement of pigs in the barns. From each barn, a total of ten Swiffer® wipes and ten drag swab samples and a negative control from each were collected simultaneously. Samples were transported on ice and processed for *Salmonella* isolation. Prevalence of *Salmonella*, serogroups and antimicrobial resistance profiles were analyzed.

Results: Salmonellae were isolated from 35 of 352 (9.9%) drag swabs and 24 of 352 (6.8%) Swiffer® wipe samples collected from swine barns ($P < 0.05$). There was a significant reduction of *Salmonella* between the pre- and post-disinfection samples: drag swab method 14.2% vs 5.6% and Swiffer® wipes 11.3% vs 2.2% respectively. *Salmonella* isolates belonged to serogroup B (39.5%), C (35.7%) and E4 (11.7%) There was no major difference in the distribution of *Salmonella* serogroups between Swiffer® and drag swab samples. Among the antimicrobial resistant isolates so far tested, multidrug resistance was observed mainly to ampicillin, chloramphenicol, streptomycin, sulphisoxazole and tetracycline (R type: ACSSuT, 23.8%) and to streptomycin, sulphisoxazole and tetracycline (R type: SSuT, 10.7%).

Significance: The findings suggest that the drag swab method results in better recovery of *Salmonella* from swine environment than the Swiffer® wipes and thus could be a useful sampling method in the monitoring of *Salmonella* in swine production environment. No qualitative difference in phenotypes of *Salmonella* were observed between the two approaches.

P3-10 Manual Excision vs. Surface Sampling Device – A Comparison of Methods for the Microbiological Sampling of Raw Ground Beef Components

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Introduction: The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) Notice 65-07 (Issued on October 12, 2007) sets forth requirements for "robust testing" (pg. 2, Attachment 5) with regard to sample collection and detection of *Escherichia coli* O157:H7 in raw ground beef components. The requirements issued by USDA-FSIS are intended as "best practices" to prevent the adulteration of non-intact raw beef products by *E. coli* O157:H7 and specify the number, weight, and size of samples to be collected for robust testing.

Purpose: The purpose of this study was to compare the sampling methods: (i) manual excision and (ii) a patented surface sampling device for collection of raw beef pieces from beef trim combo bins.

Methods: Three trim types: (i) 90/10 (visible lean/fat); (ii) 65/35 (visible lean/fat); and (iii) shank, were sampled with the two sampling methods. Manual excision was conducted by using sterile scissors to cut

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12 slices of exterior surface materials from the top of each combo. The dimensions of the slices that were manually excised were approximately 0.32 cm (1/8") thick, 10.2 cm (4") long, and 5.1 cm (2") wide. For the surface sampling device, the shaft was clamped in the chuck of a pneumatic drill and the shaving tube was inserted into each trim combo at a number of different locations and different depths while the drill was being operated. All samples were collected in sterile sampling bags and microbiologically analyzed for levels of total bacterial, total coliform, and *E. coli* counts.

Results: Recovery of bacteria from raw beef trim with the surface sampling device was higher ($P < 0.05$) than that of manual excision.

Significance: The surface sampling device is an acceptable alternative for sample collection of raw ground beef components and meets the requirements of robust testing defined by USDA-FSIS in Notice 65-07.

P3-11 Validation to EN ISO 16140 of a New Rapid Culture-Based *Salmonella* Detection Method

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Introduction: Salmonellosis continues to be significant in global public health. Traditional culture methods for the detection of *Salmonella* are time consuming, taking up to 4–5 days to complete. The Oxoid *Salmonella* Precis™ method is a new method making it possible to confirm the presence/absence of *Salmonella* spp. in less than 48 h.

Purpose: The purpose of this study was to independently validate the performance of the new *Salmonella* Precis method against EN ISO 6579:2002 according to the specifications in EN ISO 16140:2003.

Methods: The method comprises incubating the sample in a selective enrichment broth (ONE Broth-*Salmonella*) for 16–20 h, followed by plating onto a single Brilliance™ *Salmonella* plate, which is incubated for 22–26 h. Suspect colonies can be rapidly confirmed by latex agglutination. 424 samples from 6 different food categories (meat products, milk products, seafood products, egg products, animal feed and environmental samples) were analyzed to determine the relative accuracy, sensitivity and specificity of the method. Inclusivity and exclusivity tests of the *Salmonella* Precis method were conducted, using pure cultures of 40 non-*Salmonella* strains and 53 *Salmonella* strains from 38 different serovars. In addition, a collaborative study was performed by 13 laboratories to test the repeatability and reproducibility of the new method.

Results: The values of relative accuracy, sensitivity, and specificity for the *Salmonella* Precis method were statistically equivalent to those of the reference method. Characteristic colonies were detected on Brilliance *Salmonella* from all 38 different *Salmonella* serovars. Exclusivity testing showed that 38 out of the 40 non-target strains gave non-*Salmonella* characteristics on Brilliance *Salmonella* plates. The collaborative study showed a relative accuracy, sensitivity and specificity of 99.7%, 100% and 100% respectively, and demonstrated equivalence with the reference method.

Significance: The *Salmonella* Precis method has been successfully validated according to EN ISO 16140 for the detection of *Salmonella* in food and environmental samples.

P3-12 Comparison of Enrichment Media for Recovery of *Escherichia coli* O157:H7 from Meat and Environmental Samples

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Introduction: Sensitive and reliable enrichment methods are needed for detection of *E. coli* O157:H7 in foods.

Purpose: The purpose was to evaluate media for recovery of *E. coli* O157:H7 from meat and environmental samples in response to a USDA/FSIS need to decrease enrichment time from 20–24 to 15 h.

Methods: E. coli O157:H7 ATCC 43888 was used to evaluate the performance of modified *E. coli* broth containing novobiocin (mEC+n) incubated at 35°C and 42°C, with RapidChek® *E. coli* O157:H7 broth at 42°C, BioControl® mEHEC broth at 42°C, DuPont BAX® *E. coli* O157:H7 broth at 42°C, and modified Tryptone Soya Broth (mTSB) both with and without novobiocin at 42°C. The mTSB+n at 42°C was further compared to mEC+n at 35°C using ground beef, summer sausage, hard salami, cooked beef patties, beef trim, and environmental samples spiked with *E. coli* O157:H7 at 4 or 20 CFU/25 g and then incubated for 15 h.

Results: There was no statistical difference ($P < 0.05$) between mTSB+n and the commercial media using the Bactometer®, BAX® PCR assay, or standard plate counts (CFU/ml) on BHI agar. These same media had shorter Bactometer® detection times and higher percentages of BAX® positive test results than mEC+n at 35°C or 42°C. Also, more CFU/ml were recovered from mTSB+n and the commercial media after 15 h at 42°C than from mEC+n at 35°C and 42°C. Additionally, more samples tested positive with use of the BAX® PCR assay for summer sausage, hard salami, and beef trim following enrichment in mTSB+n at 42°C than from mEC+n at 35°C. However, no difference in performance was seen with cooked beef patties or environmental samples between these two media with the BAX® PCR assay.

Significance: Enrichment of contaminated meat samples in mTSB+n for 15 h at 42°C is a suitable method for recovery of *E. coli* O157:H7.

P3-13 Increased Levels of Lithium Chloride in Growth Media Eliminates the Growth of *Enterococcus* spp. during Recovery of *Listeria* spp. from Environmental Samples

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Introduction: Listeria spp. are ubiquitous bacteria that are employed as measures of hygiene in the food processing environment. Broth-based methods for detection of *Listeria* spp. have been developed, but the growth of enterococci often leads to false positive test results.

Purpose: This study evaluated the supplementation of *Listeria* growth media with increased concentrations of lithium chloride (LiCl) as a way to suppress the growth of *Enteroccocus* spp.

Methods: Tryptic Soy Broth (TSB) was supplemented with concentrations of LiCl ranging from 5 g/L to 12 g/L. Twenty-four h growth curves of *Listeria monocy-*

togenes strains J1-177, 10403S, and *L. innocua* strain W1-139, and *Enterococcus faecalis* strains EN 1, 2, and 3 were conducted in duplicate, in the LiCl supplemented TSB.

Results: The addition of up to 8 g/L LiCl increased the lag phase of all *E. faecalis* strains by as much as 15 h (compared to the control). In contrast, no differences in *L. monocytogenes* growth were observed. At LiCl concentrations above 8 g/L, the *E. faecalis* strains showed minimal growth (OD600 nm of 0.1) after 24 h, while all *Listeria* strains grew, although there was an increase in the lag phase of strain 10403S by 1 h as compared to the control. Individual stainless steel coupons were inoculated with 10^6 CFU/ml of *L. monocytogenes* J1-177 or *E. faecalis* EN 3. After drying, the coupons were individually swabbed and tested with a phosphatidylinositol-specific phospholipase C (PI-PLC) detection assay. After a 13 h incubation step in TSB with 12g/L LiCL, the absorbance of the *L. monoctyogenes* sample was OD600 nm 1.09, while the absorbance of the *E. facaelis* sample was 0.06. The presence of PI-PLC confirmed the growth of *L. monocytogenes*.

Significance: These data suggest that increasing the concentration of LiCl in *Listeria* growth media may effectively suppress the growth of *Enterococcus* spp.

P3-14 Comparison of Supplements to Enhance the Recovery of Thermally-Injured *Salmonella* from Liquid Egg White

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Introduction: The recovery of *Salmonella* from liquid egg white (LEW) is complicated by thermal and innate LEW antimicrobial-induced injury. Numerous supplements have been reported to promote the recovery of injured bacteria.

Purpose: The purpose of this study was to determine the efficacy of twelve media supplements in affecting the recovery of heat-injured *Salmonella* from LEW.

Methods: A five-strain composite of *Salmonella* was inoculated in LEW at 7.72 log CFU/ml, heated at 53.3°C for 4 min., inducing ca. 2 log CFU/ml inactivation, and serial dilutions were plated on media with or without supplements.

Results: Greater numbers of *Salmonella* ($P < 0.05$) recovered with the addition of 1 g/L ferrous sulfate ($FeSO_4$) to Tryptic Soy Agar (TSA) than with the addition of any other supplement, except for 0.5 or 1 g/L 3'3'-thiodipropionic acid. Addition of 1 g/L sodium pyruvate ($CH_3COCOONa$), or 6 g/L yeast extract plus 1g/L $CH_3COCOONa$ to TSA supported greater resuscitation than unsupplemented TSA or TSA supplemented with 0.01 or 0.1 g/L N-propyl gallate, 10 g/L activated charcoal, 0.1 g/L KMnO4, or 50 mg/L ethoxyquin. Even fewer numbers of *Salmonella* ($P > 0.05$) recovered on solidified Fluid Thioglycollate medium or on TSA with addition of the following compounds, ranked in order of performance: 1 g/L EDTA > 50 g/L sodium thiosulfate ($Na_2S_2O_3$) > 15 g/L NaCl. The remaining supplements supported recovery of equivalent numbers of *Salmonella*, which were fewer cells than recovered on TSA with 1 g/L $FeSO_4$ yet greater populations than recovered on TSA with 50 mg/L ethoxyquin. These compounds included 3.5 g/L or 6 g/L $CH_3COCOONa$, 1 or 5 g/L activated charcoal, 0.1 or 0.01g/L $FeSO_4$, 6 g/L yeast extract, 1 or 0.5 g/L EDTA, 5 or 0.5mg/L ethoxyquin, 0.5 or 5 g/L $Na_2S_2O_3$, 1, 0.1, or 0.01 g/L ferric ammonium citrate, as well as unsupplemented plate count agar or TSA.

Significance: These data suggest that media supplementation with $FeSO_4$, 3'3'-thiodipropionic acid, or $CH_3COCOONa$ may aid in recovering sublethally injured *Salmonella* from LEW.

P3-15 DSC Comparison of Selective Media for Detection of *Escherichia coli* O157:H7 in Ground Beef and Radish Sprout

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Introduction: Escherichia coli O157:H7 is an important foodborne pathogen, causing bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Various selective media have been used for detection of *E. coli* O157:H7.

Purpose: The purpose of this study was to compare two selective media (SMAC, CT-SMAC) for isolation of *E. coli* O157:H7 from artificially inoculated raw ground beef and radish sprout, with nalidixic acid resistant *E. coli* O157:H7.

Methods: Ground beef and radish sprout (500 g each sample) were inoculated with nalidixic acid-resistant *E. coli* O157:H7 at 20 CFU and 1400 CFU, respectively and divided into 20 samples (25 g/sample). Three negative and one positive controls were included in each experiment. All samples (25 g each) were mixed with mTSB (225 ml/sample) and incubated at 37°C for 24 h. After the enrichment, broth cultures were streak plated onto 3 selective media, Sorbitol MacConkey Agar (SMAC), Sorbitol MacConkey Agar with cefixime and tellulite (CT-SMAC), and Sorbitol MacConkey Agar with nalidixic acid (NAL-SMAC) as the gold standard. After incubating at 35–37°C for 18–24 h, two suspicious colonies from the selective media were picked and plated on Tryptic Soy Agar with 0.6% yeast extract (TSAYE) and incubated at 35°C for 18–24 h, followed by serological confirmation.

Results: The rate of positive samples out of twenty samples tested were 70% (14/20) from SMAC, 65% (13/20) from CT-SMAC, 75% (15/20) from NAL-SMAC in ground beef and 10% (2/20) from SMAC, 35% (7/20) from CT-SMAC, 35% (7/20) from NAL-SMAC in radish sprout.

Significance: There was no significant difference in the number of positive samples between SMAC and CT-SMAC in ground beef, while more positive samples were detected in CT-SMAC than SMAC in radish sprout. It appears that CT-SMAC is superior to SMAC for detecting *E. coli* O157:H7 in food samples with high background flora, such as radish sprout.

P3-16 Efficacy of a Chromogenic Plating Medium for Detecting *Listeria* Species from Environmental Samples

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Introduction: The chromogenic plating medium R & F *Listeria* sp. *Listeria monocytogenes* Plating Medium (LSPM) differentiates *Listeria monocytogenes* as blue-violet and nonpathogenic *Listeria* sp. as pink colonies on a single plate against a white background.

Purpose: The chromogenic plating medium R & F *Listeria* sp./*Listeria monocytogenes* Plating Medium (LSPM) differentiates *Listeria monocytogenes* as blue-violet and nonpathogenic *Listeria* sp. as pink colonies on a single plate against a white background.

Methods: Enrichment cultures from environmental sponges obtained from multiple production sources were rapid-tested (Reveal *Listeria* Test System) for the presence of *Listeria* sp. Presumptively positive samples were streaked on LSPM, on Modified Oxford Medium (MOX), and on a FDA approved chromogenic plating medium (R & F *Listeria monocytogenes* Plating Medium [LMPM]). The percentages of individual *Listeria* sp. recovered were identified by Micro-ID® Listeria (Remel).

Results: The percentages of individual *Listeria* sp. recovered from 80 samples on LSPM and MOX were similar, with respective values of 60.0/61.3 for *L. monocytogenes*; 23.8/23.8 for *L. innocua*; and 3.8/3.8 for *L. grayi*. For samples with mixed *Listeria* sp., values were 5.0/0.0, respectively. Potential false positives isolated as pink colonies on LSPM included *Cellulomonas*, *Lactobacillus*, and *Stomatococcus*, whereas *Bacillus*, *Cellulomanas*, *Enterococcus*, *Kurthia*, and *Micrococcus* were isolated from MOX plates. Atypical alterations in pleiomorphic characteristics (loss of tumbling or catalase production, or both) were observed in 35 confirmed direct isolations from MOX, but not from LSPM. The mono-culture nature of the samples tested, plus the chromogenic responses on LSPM, allowed single colony isolations in most cases to be sufficient for differentiation into *L. monocytogenes* or nonpathogenic *Listeria* sp. Conversely, direct differentiation was precluded on MOX plates because all *Listeria* appear as black/brown colonies due to esculin hydrolysis.

Significance: LSPM offers an accurate, rapid, and direct plating method for differentiating the presence or absence of *L. monocytogenes* and other *Listeria* sp. in naturally contaminated environmental samples.

P3-17 Recovery of *Listeria monocytogenes* from Pasteurized Liquid Egg Products

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Introduction: Pasteurized liquid egg products (PLEP) are sold for both commercial and retail uses. Because it can grow at low temperature, *Listeria monocytogenes* has the potential to be an important pathogen in these types of products.

Purpose: USDA-FSIS-OPHS is interested in testing its existing methods for screening and confirmation of *L. monocytogenes* in pasteurized liquid egg products. A secondary goal of the study was to determine if secondary enrichment in Fraser Broth could be replaced with MOPS-BLEB without loss of recovery.

Methods: In Phase 1, the limits of detection (LOD) in four types of PLEP were determined: Egg whites (with or without added Ingredients); whole eggs or yolks (with < 2% added ingredients other than salt or sugar); whole eggs with added yolks, or whole egg blends (with > 2% added ingredients other than salt or sugar); and whole eggs or yolks (with > 2% salt or sugar added). In Phase 2, the recovery of 4 different serotypes of *L. monocytogenes* (1/2a, 1/2b, 3b, 4b) inoculated at high and low levels were determined. Phase 3 was a three-lab method validation with spiked samples.

Results: Recovery varied according to the composition of the egg product tested. Of the matrices analyzed in Phase 1, whole egg had the lowest LOD of 4.04×10^{-2} CFU/g; 1.04×10^{1} CFU/g was required to detect LM in egg whites, which represented the highest LOD. In Phase 2, serotype did not affect recovery in any egg product tested. Phase 3 resulted in similar rates of recovery for each method in each lab. Use of MOPS-BLEB enrichment for cultural confirmation of screen positive samples was found to be as effective as Fraser Broth.

Significance: The data demonstrated that: (1) the FSIS MLG Chapter 8A screening protocol would work on a variety of liquid egg samples, (2) use of the screen and MOPS-BLEB secondary enrichment is comparable to Fraser Broth secondary enrichment, (3) recovery rates for *L. monocytogenes* can be expected to vary depending on the liquid egg composition, and (4) use of the MLG Chapter 8A screening method for liquid egg products is fit for the purpose.

P3-18 Comparison of Four Compact Dry Plate Methods against Standard (ISO) Methods for the Enumeration of *Enterobacteriaceae*, Coliforms and *Escherichia coli* in Foods during a MicroVal EN ISO 16140 Validation

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Introduction: Compact Dry (Nissui Pharmaceutical Co. Ltd; supplied by Hyserve Gmbh & Co. KG) are Ready-to-Use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the center of the self-diffusible medium. This study presents results of comparing the Compact Dry ETB, CF and EC methods against the respective standard (ISO) methods for the enumeration of *Enterobacteriaceae* (CD-ETB), total coliforms (CD-CF) and *Escherichia coli* and total coliforms (CD-EC) in a range of foods.

Purpose: Comparison of four Compact Dry methods against the respective standard (ISO) methods, using validation protocol EN ISO 16140.

Methods: Compact Dry methods were performed in accordance with the manufacturers' instructions. The standard methods used were ISO 21528-2:2004 (*Enterobacteriaceae*) ISO 4832:2006 (coliforms) and ISO 16649-2:2001 (*E. coli*). The method comparison study compared each method using naturally/artificially contaminated raw ground beef, cooked chicken, lettuce, milk powder and frozen fish. The interlaboratory study involved 12 laboratories in 5 countries testing artificially contaminated milk samples. Results were analysed using the principles of EN ISO 16140.

Results: Calculated correlation coefficients (R-Sq) were 0.970 (CD-ETB) for *Enterobacteriaceae*, 0.967 (CD-CF) and 0.959 (CD-EC) for coliforms and 0.973 (CD-EC) for *E. coli* (all foods combined). Interlaboratory study results revealed no evidence of differences in reproducibility. Compact Dry plate method results were equivalent to the respective reference methods for the enumeration of *Enterobacteriaceae*, coliforms and *E. coli*.

Significance: Compact Dry plate methods gave comparable results to the reference methods.

P3-19 Evaluation of ChromID *sakazakii* Medium (ESPM) for the Recovery of *Enterobacter sakazakii* from Several Food and Environmental Samples

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Introduction: Enterobacter sakazakii was described as a bacterial species in 1980. It has been implicated

in a severe form of neonatal meningitis with a high mortality rate. It is reported that many newborns with *E. sakazakii* meningitis die within days of infection, and that the case-fatality rates vary between 40 and 80%. While a reservoir for *E. sakazakii* bacteria is unknown, reports have suggested that powdered milk-based infant formula may be a vehicle for infection.

Purpose: The aim of this study was to evaluate the performances of chromID *sakazakii* medium against two other chromogenic media for the recovery of *E. sakazakii* strains from newborn food and environmental samples.

Methods: Sensitivity, specificity and selectivity of three media were assessed on 65 newborn food and 8 environmental samples. Among these 65 food samples, 30 of them were artificially contaminated by a low level of 10 strains of *E. sakazakii* (approximately 10 to 100 CFU/g). After a pre-enrichment step in BPW followed by an enrichment in mIST + vancomycin broth, recovery of *E. sakazakii* strains was assessed on three chromogenic media.

Results: Data demonstrated that the recovery of *E. sakazakii* species was equivalent on the three media after 24 h incubation. Indeed, in all the 30 artificially contaminated samples, it was recovered on the chromogenic media with colored colonies. On the 65 natural food samples, three contained *E. sakazakii* strains which were isolated on the three chromogenic media. For the 8 environmental samples, one of them was also positive and found on the three media. Concerning the growth of contaminant flora, chromID *sakazakii* is found to be the more selective medium. On the 73 samples tested, 2 of them grew on chromID *sakazakii* against 5 on the two other media.

Significance: These data suggested that chromID *sakazakii* medium is very useful for the isolation of *E. sakazakii* strains from food and environmental samples. Its high sensitivity, specificity and selectivity enable an accurate recovery of *E. sakazakii* after 24 h incubation at 41.5°C. The color of colonies facilitates the reading of this medium and the pre-orientation of *E. sakazakii* strain.

P3-20 Suitability of Modified Eosin Methylene Blue Agar for Recovering Heat-Injured *Escherichia coli* O157:H7 and *Salmonella* Serovars from Cooked Meat Products

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Introduction: Growth of heat-injured pathogens may be inhibited by components of selective media. Therefore, plating on selective media can lead to overestimating thermal process lethality in meat-processing validation studies. Previous work on the recovery of heat-stressed *E. coli* O157:H7 from ground beef showed the potential for enhanced cell recovery by use of direct plating on modified eosin methylene blue agar (MEMB).

Purpose: Our first objective was to compare recovery of *E. coli* O157:H7 from cooked ground beef samples plated with direct and injury-repair overlay-plating methods with MEMB and Sorbitol MacConkey Agar (SMAC). Our second objective was to compare recovery of *E. coli* O157:H7 and *Salmonella* serovars from heated/dried ground-and-formed beef jerky plated directly on MEMB and overlay-plated using xylose-lysine-desoxycholate agar (XLD).

Methods: MEMB is lactose-free EMB with added sorbitol and sodium chloride to allow differentiation between sorbitol-negative *E. coli* O157:H7 and *Salmonella*. Ground beef was inoculated with 5 strains of *E. coli* O157:H7 before moist-heating. A separate inoculum containing 5 strains each of *E. coli* O157:H7 and *Salmonella* was added to ground beef made into beef jerky. Samples were taken throughout heating of both products and analyzed. Injury-repair tests consisted of plating on non-selective media followed by an overlay of MEMB, SMAC, or XLD, as appropriate. Log CFU values were obtained with each method.

Results: Direct-plating on MEMB recovered significantly more *E. coli* O157:H7 and *Salmonella* than SMAC direct-plating or XLD overlay methods, respectively ($P < 0.05$). Using MEMB as an injury-repair overlay medium recovered significantly more *E. coli* O157:H7 than direct plating on SMAC ($P < 0.05$). While no statistically significant difference was found between MEMB and SMAC overlay-plating, there was a consistent trend of greater recovery on MEMB.

Significance: By increasing recovery of *E. coli* O157:H7 and *Salmonella*, use of MEMB in direct or overlay plating will improve accuracy of estimating meat thermal process lethality.

P3-21 Comparison of Eosin Methylene Blue Agar with Other Selective Media for the Isolation and Presumptive Identification of *Escherichia coli*

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Introduction: Eosin Methylene Blue (EMB) agar is an official selective agar used to isolate *Escherichia coli* in Korea. However, the selection capability of EMB to isolate *E. coli* is so low that it is hard to distinguish *E. coli* from other related gram negative enteric bacteria.

Purpose: The aims of this study were to identify possible selective media for the efficient isolation of *E. coli* and to evaluate their selectivity.

Methods: The same 8 sets of 12 plates containing 12 different selective agars were used, and 8 different types of strains were prepared: 2 different GUD-positive *E. coli* strains (ATCC 25922 and ATCC 10536), 1 non-GUD-negative *E. coli* strain (ATCC 43895), and 5 non-*E. coli* strains which were *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 10031), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 29737), and *Yersinia enterocolitica* (ATCC 9610). Each of these 8 different strains was streaked onto a set of plates containing 12 different selective agars and incubated at 35°C for 24 h. After incubation, the colony color of the selective agar on each plate was observed. The selective agar showing the best selection capability was chosen. Then, *E. coli* detection capabilities of both EMB and the chosen agar were evaluated with food samples: beef, pork, chicken, spinach, bean curd and red bean bread. Lauryl Sulfate Broth with MUG was used in the comparison test.

Results: On EMB, a metallic green colony was observed for *E. coli* (ATCC 25922) similar to a *Salmonella* colony; however, a violet colony was observed for *E. coli* (ATCC 10536). In the case of Chromocult Tryptone Bile x-glucuronide (CTBX), blue-green colonies were observed for 2 different GUD-positive *E. coli* strains (ATCC 25922 and ATCC 10536) whereas other gram negative enteric bacteria showed as white colonies. The comparison test with food samples showed that 0 and 83% of presumptive colonies were positive for *E. coli* on EMB and CTBX, respectively. The result indicated that CTBX was quite selective to detect *E. coli*, in contrast to EMB.

Significance: CTBX showed better selectivity and specificity for the isolation of *E. coli* than EMB. CTBX could be recommended as an alternative medium for the isolation and presumptive identification of *E. coli*.

P3-22 The Application of Immunomagnetic Separation in Combination with ALOA *Listeria* Chromogenic Agar for the Isolation and Identification of *Listeria monocytogenes* in a Variety of Foods

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Introduction: Listeria monocytogenes is a foodborne pathogen that has a high mortality rate. Improved methods are needed for isolation and identification of this pathogen in foods.

Purpose: The use of an immunomagnetic separation method (IMS) in combination with ALOA *Listeria* chromogenic agar was investigated for the isolation and identification of *Listeria monocytogenes* from a variety of foods.

Methods: Raw meat, processed meat, fish and seafood, cultured and non cultured dairy, egg and egg products, and produce were inoculated at a level of 1, 10, and 100 CFU/25 g with *L. monocytogenes* ATCC 19115 and stressed at 4°C for 24 h prior to testing. IMS was performed as described by Dynal Biotech (Oslo, Norway), using a BeadRetriever Automated IMS system (Dynal Biotech) followed by spread plating of 25 µL each onto ALOA *Listeria* chromogenic agar (AES Chemunex) and Modified Oxford agar, a conventional selective agar. As a comparison method, the same sample enrichments were tested as described by the BAX Q7 *Listeria monocytogenes* PCR Assay (DuPont).

Results: Results showed that at the 10 and 100 CFU/25 g inoculum level, both methods showed a 100% correlation. However, at the 1 CFU/25 g inoculum level where fractional recovery was observed, 70 positive samples of 110 were reported by the BAX *L. monocytogenes* PCR Assay compared to 75 positive samples by the IMS-ALOA *Listeria* chromogenic agar combination. Analysis of the BAX secondary enrichment by standard culture methods confirmed the presence of *L. monocytogenes,* indicating that these BAX PCR results were false negatives.

Significance: The IMS-ALOA *Listeria* chromogenic agar combination was used successfully in isolating and identifying *L. monocytogenes* from seeded samples in the same time frame (48 h) and in some cases faster (in selected food matrices) than a screening result is obtained by use of the BAX PCR *Listeria monocytogenes* Assay. Further work, including the selection of a single enrichment medium, optimization of incubation conditions, alternate IMS protocols, and further evaluation of chromogenic agars, as well as testing of naturally contaminated samples, is being pursued.

P3-23 DSC Addressing Potential Contaminants in Soil for the Study of Pathogenic *Escherichia coli* O157 and O8 Strains

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Introduction: Foodborne illness associated with leafy greens has illustrated the need for information describing the transmission of pathogens via contaminated soil amendments. A realistic means of studying the fate of pathogens, simulating natural conditions, proved difficult because of high levels of bacterial and fungal soil organisms.

Purpose: The purpose of this study was to establish methods to assess the fate of pathogenic *Escherichia coli* strains (avian pathogenic (APEC) O8, APEC O157, 2006 spinach and lettuce outbreak isolates) in biosolids, manure and soil. APEC strains caused damage to the DELMARVA poultry industry.

Methods: Five-gram samples of poultry litter, dairy manure, or treated biosolids were inoculated with a cocktail of *E. coli* (10^6 CFU/g) and mixed into 45 g of soil on which spinach seedlings were sprouted. Initial counts were plated on Tryptic Soy Agar (TSA) containing nalidixic acid (NA); however, this medium proved unsuitable because of growth of contaminating organisms. In order to enumerate *E. coli,* several media types were explored (xylose-lysine-deoxycholate, Levine's Eosin Methylene Blue (L-EMB), MacConkey with sorbitol (SMAC) hektoen enteric and SMAC containing NA). The efficacy of *E. coli* O157 antibody-coated magnetic Pathatrix beads to recover APEC strains from soil was also evaluated.

Results: L-EMB proved most inhibitory to competitive soil microflora of the media studied with recovery of 1.5×10^6 for APEC O8 and O157. Leafy green O157 outbreak strain counts varied with soil amendment, 1.0×10^7 in poultry litter and 1.0×10^8 in anaerobically digested biosolids. Gram stains and microscopy confirmed presumptive *E. coli* counts. Additionally, APEC O157 (1.3×10^6 CFU/g) and O8 (3.0×10^6 CFU/g) could be recovered from soil inoculated with 10^8 CFU/g, using *E. coli* O157 PATHATRIX beads and plated onto TSA-NA without the need for further selective media.

Significance: This work provides novel techniques to evaluate the survival and transport of pathogenic *E. coli* within different manure and biosolids applied during cultivation of spinach.

P3-24 Comparison of Colony Lysis Procedures for *Listeria monocytogenes* PCR

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Introduction: Previous studies in this laboratory, using a PCR method for *Listeria monocytogenes,* demonstrated poor test sensitivity that arose from insufficient DNA template. In the method, individual colonies of *Listeria* were heat-lysed prior to PCR amplification. The small size of *L. monocytogenes* colonies grown on selective agar, combined with incomplete cell lysis, was hypothesized to contribute to the poor test performance.

Purpose: The purpose of this study was to enhance the efficiency of DNA extraction from a single colony of *L. monocytogenes* and thereby increase the overall sensitivity of our PCR method.

Methods: L. monocytogenes (ATCC 19114) was grown for 48 h on three different selective agars: Oxford agar, Palcam agar and a *Listeria* chromogenic agar. Individual, well isolated colonies were lysed, using either Procedure A (heat in 0.1 ml water at 95°C for 5 min) or Procedure B (heat in 0.1 ml buffered 1% Triton X-100 at 100°C for 10 min). These extracts were tested by our PCR method and results for each lysis procedure and agar type were compared.

Results: A total of 200 colonies of *L. monocytogenes* were picked from selective agar and lysed by Procedure A or Procedure B prior to PCR testing. Procedure B demonstrated greater test sensitivity than Procedure A (97% vs. 68%) for the PCR confirmation of

L. monocytogenes, with no significant difference in test sensitivity based on the agar type.

Significance: Extensive heat and the presence of detergent were demonstrated to increase the efficiency of DNA extraction from *L. monocytogenes* and were important for achieving high sensitivity in *Listeria* colony PCR.

P3-25 A Highly Sensitive Real-Time PCR Assay for the Detection and Identification of *Campylobacter jejuni* from Retail Broiler Samples

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Introduction: Traditional detection methods for *Campylobacter jejuni* require the enrichment of the food samples to achieve a high number of bacterial cells. Efforts to reduce the detection time by testing enrichment broths with polymerase chain reaction (PCR) assays have been mainly unsuccessful.

Purpose: The objective of this work was to develop a real-time PCR protocol for the identification of *C. jejuni* from retail broiler samples.

Methods: A double labeled hydrolysis probe and a primer set were designed from the conserved region of the benzoyglycine amidohydrolase (hippuricase) gene of *C. jejuni*. The specificity of the assay was then tested with several *Campylobacter* strains and other Gram-negative bacteria.

Results: The PCR protocol was highly specific, and only DNA from *C. jejuni* strains was amplified. Quantitative detection was conducted after generation of the standard curve with serially diluted DNA extracted from pure culture, which was found to be linear over 9 log units, with a standard curve correlation coefficient of 0.998. The detection limit for the current assay is 4.6 colony forming unit (CFU) per milliliter in pure cultures. This real-time PCR method was also used to detect *C. jejuni* from naturally and inoculated retail samples; the detection limit of this assay for enriched samples was 100 CFU per milliliter.

Significance: This assay may reduce the time for detection of *C. jejuni* in retail broiler samples.

P3-26 Validation of a PCR-Based Protocol for the Rapid Detection of *Salmonella* from Environmental Surfaces

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Introduction: Recent product recalls and outbreaks have demonstrated the importance of detecting and controlling *Salmonella* in the food-manufacturing environment. Different surfaces in food processing facilities can act as reservoirs that allow for the persistence of this pathogen in manufacturing environments and contamination of finished product.

Purpose: Sensitive, well validated methods for the detection of environmental *Salmonella* are critical to controlling the risk associated with this organism. This study tested the efficacy of screening laboratory contaminated stainless steel surfaces, using the BAX® System PCR assay for the detection of *Salmonella*.

Methods: This study tested twenty-five 4 × 4 inch stainless steel surfaces. Twenty were inoculated with 1 ml of a 60 CFU/ml cocktail *Salmonella* culture (*Salmonella* Senftenberg, Tennessee (2 strains), Enteritidis (2 strains), Dessou, and Chingola) while the remainder was inoculated with the culture diluent (10% non-fat dry milk). The samples were dried at room temperature for 24 h and collected with a sponge. The sponges were transferred into lactose broth and incubated at 35°C. At 20 h an aliquot was taken for direct testing by the new assay. Corresponding aliquots were taken for non-selective re-growth in BHI for 5 h at 35°C followed by BAX® assay and for selective secondary enrichment in TT and SC broths and plating on XLD and HE agars.

Results: The system PCR assay for *Salmonella* without re-growth detected 16 out of 17 culture-confirmed positive samples. The new assay after BHI re-growth detected all 17 samples that would ultimately be confirmed positive for the presence of *Salmonella*.

Significance: This study indicated that the system PCR assay for *Salmonella* detection is a rapid, sensitive method for the detection of this pathogen from stainless steel environmental surfaces. Test kit results demonstrated 100% correlation with the culture-based detection method.

P3-27 High Throughput *Salmonella* Testing Using a 10 Sample (Post Pre-Enrichment) Pooling Strategy Linked to Re-Circulating IMS and Real Time PCR

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Introduction: A Re-circulating Immunomagnetic Separation technique (RIMS) has previously been AOAC-RI validated for the rapid isolation of low level *Salmonella* contamination (110 CFU per sample), from pooled samples consisting of a combination of five different individual samples.

Purpose: The purpose of this study was to evaluate the feasibility of using the RIMS system for the analysis of pooled samples; consisting of a combination of ten individual post pre-enriched samples. A variety of food matrices were investigated.

Methods: Food matrices (up to 75g) (including chocolate, cocoa products, milk powders and dried soups) were weighed and diluted as appropriate to sample type. A single sample from each sample type was inoculated with low levels of *Salmonella* (110 CFU/sample) while all other samples remained uninoculated. All samples were incubated for 18 h at 37°C after which aliquots from 10 individual samples were combined (1 inoculated: 9 uninoculated) creating a 10-pooled sample. A 30 min RIMS capture selectively concentrated the target bacteria; detection of *Salmonella* was achieved using real time PCR and selective agar plating techniques.

Results: The data showed that RIMS can successfully isolate *Salmonella* serovars from 10-pooled samples in a variety of different food matrices. Detection of the low level inoculums was achieved using either Real Time PCR or selective agar plates.

Significance: The RIMS 10-pooling method enables the detection of *Salmonella* from up to 10 × 75 g of sample using only one PATHATRIX consumable and PCR reagent. This is an extremely cost effective and labour efficient method of pathogen testing allowing laboratories greater sample throughput together with significant cost savings.

P3-28 PATHATRIX Recirculating Immunomagnetic Separation – A Unique and Versatile System for the Rapid Detection of Foodborne Pathogens in Leafy Produce, Herbs and Spices

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Introduction: Ready-to-eat leafy produce, herbs and spices present significant challenges for the detection of foodborne pathogens such as *E. coli* O157, *Salmonella* and *Listeria* spp. Fresh produce has been implicated in a significant number of foodborne disease outbreaks during the past decade. Herbs and spices are used ubiquitously as seasoning in a variety of foods, some of which have been linked to cases of salmonellosis and listeriosis.

Purpose: This study describes the development of re-circulating immunomagnetic separation (RIMS) methods that allow: post growth sample pooling; analysis of large (1875 g) spice samples for *Salmonella* or *Listeria*; and simultaneous same-day isolation of *E. coli* O157:H7 and *Salmonella* from fresh produce.

Methods: Fresh produce samples (25 g) were diluted 1:9 with pre-warmed Brain Heart Infusion broth. Spice samples (375 g) were diluted 1:9 with Half-Fraser broth or Buffered Peptone Water supplemented as appropriate with 0.5% potassium sulfite with respect to the target pathogen and sample type. One sample in each set of 5 received an appropriate inoculum of either: *E. coli* O157 *and Salmonella* (fresh produce); *Listeria* or *Salmonella* (spices) to mimic low-level contamination (1–10 CFU/sample). After the appropriate pre-enrichments aliquots from each set of 5 samples were pooled to create single samples. RIMS was used to capture the target pathogen from the samples. *E. coli* O157:H7 and *Salmonella* were isolated simultaneously from pooled pre-enriched fresh produce using a blend of dual IMS beads. Detection of the target pathogens was achieved using both real time PCR and selective agar plating.

Results: The isolation and detection of *E. coli* O157:H7 and *Salmonella* (simultaneously) in pooled (125 g) fresh produce samples was comfortably achieved within a working day (< 8 h) using RIMS linked to RT-PCR. The data also shows that RIMS linked to RT-PCR can be successfully used to isolate and detect *Listeria* spp. and *Salmonella* in post growth pooled spice samples when target pathogens are initially present at low levels (1–10 CFU in 1875 g). Recovery of the target pathogens on selective agar plates confirmed the PCR results in all cases.

Significance: The RIMS pooling methods offer high sample throughput, significant cost savings and enhanced pathogen detection without loss of sensitivity as sample pooling is carried out after pathogen growth but prior to PCR detection. Full traceability of the original sample is maintained until results are confirmed.

P3-29 DSC MPN Determination of *Salmonella* Levels in Naturally-Contaminated Raw Almond Kernels with Two Sample Preparation Methods and Comparison of the Isolates Using Pulsed Field Gel Electrophoresis

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Introduction: For particulate foods such as almonds, uneven distribution, especially clumping of the pathogen, may impact interpretation of MPN results. Characterization of *Salmonella* isolates from almonds, beyond serotype, is important in understanding potential sources of contamination.

Purpose: The purpose was to determine the levels of *Salmonella* in naturally-contaminated raw almonds, using two sample preparation methods, and to characterize multiple serovar isolates by Pulsed Field Gel Electrophoresis (PFGE).

Methods: Raw almond kernels were collected from almond processors throughout California during the 2006 harvest. Samples (100 g) were enriched for *Salmonella* and levels of the organism were determined for positive samples by three-tube MPN (25 g, 2.5 g, 0.25 g), using two different methods of sample preparation. Almonds were either divided into subsamples prior to blending and enrichment (Method A) or samples were blended in enrichment broth prior to preparation of subsamples (Method B). All *Salmonella* isolates were serotyped and serovars isolated more than once were further characterized by PFGE, using restriction enzyme Xba1.

Results: Salmonella was recovered from 30 of 1,899 (1.6%) almond samples but was not isolated (MPN < 1.2/100 g) upon retesting of 19 of 30 (Method A) or 23 of 29 (Method B) positive samples. When detected, levels were 1.4 to 15.5 MPN/100 g (average 2.3 MPN/100 g) or 1.4 to 18.3 MPN/100 g (average 2.0 MPN/ 100 g), using Method A or B, respectively. A total of 62 isolates were recovered from the original and MPN subsamples, of which 10 serotypes were identified. Multiple isolates of serovars, Muenchen (20), Montevideo (13), Enteritidis (5) Newport (4), Agona (4), and Typhimurium (3) were recovered. PFGE patterns were identical within paired samples (original and MPN isolates) but differed among individual almond samples.

Significance: Sample preparation method did not impact MPN levels determined for almond kernels. Different PFGE patterns for isolates of the same serovar suggest multiple rather than point source contamination.

P3-30 Detection of the *Escherichia coli* FLICh7 Gene with Real-Time PCR

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Introduction: Escherichia coli O157:H7 is a foodborne pathogen that causes hemolytic-uremic syndrome and hemorrhagic colitis. With culture-based methods, positive identification of *E. coli* O157:H7 is made with biochemical tests to identify an isolate as *E. coli* and serological confirmation of the O157 and H7 antigens by latex agglutination using specific antisera. However, under certain conditions, some *E. coli* O157:H7 isolates can appear to be non-reactive with H7 antisera and may require multiple passages on motility medium to restore H7 antigenicity.

Purpose: We compared latex agglutination with a real-time PCR test to detect the presence of the H7 antigen or fliCh7 gene, respectively, in *E. coli* O157 isolates.

Methods: Sixty-one *E. coli* strains, including 5 O157:H7 reference strains, non-O157 reference strains that were either H7+ (2) or H7- (17), and 37 O157:H7 strains isolated from meat, of which 10 strains were involved in outbreaks. The O157:H7 strains isolated from meat were streaked onto sheep blood agar and incubated at 35°C for 18 h, and then analyzed in parallel by a commercial latex agglutination test and by

real-time PCR using primers and probes targeting the fliCh7 gene.

Results: The real-time PCR assay targeting the *E. coli* fliCh7 gene showed 100% agreement with the H7 status reported for reference strains and *E. coli* O157:H7 meat isolates. The latex agglutination test results agreed with the H7 status reported for the *E. coli* O157:H7 isolates associated with the reference strains, with one exception (an *E. coli* O117:H7 strain); however, 51% (19/37) of the *E. coli* O157:H7 meat isolates tested negative for the H7 antigen by latex agglutination.

Significance: The real time PCR test can be used to confirm *E. coli* O157:H7 strains that produce negative H7 agglutination test results.

P3-31 Effective Procedures Independent of Serotype to Detect Shiga Toxin-Producing *Escherichia coli* and Surveillance on Beef

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) infections have occurred worldwide, although the major serotype of STEC is O157 in most countries. Few procedures have been established for non-O157, although many effective methods for O157 have been developed by many researchers and companies. The scarcity of methods for the isolation of non-O157 STEC makes it difficult to decide on the proper course for reducing or preventing STEC infections.

Purpose: To detect various serotypes of Shiga toxin-producing *Escherichia coli* (STEC) in food, methods independent of serotyping were investigated in the present study.

Methods: A surface (25 g) from each of 720 beef loaf samples in 2005–2007 was incubated in modified EC broth without bile salts at 25°C for 2 h, and then bile salts and novobiocin were added, followed by incubation at 42°C for 18–20 h. Enrichment culture was tested in a loop mediated isothermal amplification (LAMP) assay targeting the Shiga toxin (ST) gene. LAMP assay positive dilutions were plated onto selective media. After incubation, suspension of a colony or some colonies was tested in LAMP assay. Positive suspension was diluted and plated onto selective media. The procedure was repeated. LAMP positive colony was confirmed as STEC and serotype.

Results: As a result of surveillance in beef, 11 of 720 samples (1.5%) tested positive for ST gene by LAMP assay. We were successful in the isolation of STEC from the enrichment culture of 11 samples by the newly established procedure. Serotype O8, O128 and O-untypable STEC were isolated from the samples. In this study, we established effective procedures for isolating STEC independent of serotype in combination with rapid and sensitive methods that included targeting the ST gene and plating onto selective agar media for *E. coli*.

Significance: The present, newly established procedure makes it possible to isolate STEC, including O145, O103, O111 and O26, as major serotypes of *E. coli* in food and identify the foods associated with foodborne infections.

P3-32 DSC Sensitive and Direct Detection of *Salmonella enterica* in Chicken Rinse by Combined Immunomagnetic Separation (IMS) and Quantitative Real-Time PCR (qPCR) with an Internal Amplification Control (IAC)

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Introduction: Development of near Quantitative Real-Time PCR methods (qPCR) to detect foodborne pathogens requires strategies to separate and concentrate the target from the food matrix and assure the absence of inhibitor-related false negative results.

Purpose: To develop a sensitive, robust, and reliable qPCR-based method for the direct detection of low numbers of *Salmonella* in chicken rinse samples.

Methods: A homologous internal amplification control was designed and tested for its ability to be reliably amplified in the presence of the target without impacting assay detection limits. An indirect Immunomagnetic Separation (IMS) method that used polyclonal goat anti-*Salmonella* antibodies and magnetic bead-bound rabbit anti-goat antibody was used to capture *Salmonella*. The IMS protocol was applied to chicken rinse samples seeded with various concentrations of *Salmonella* (10^0–10^5 CFU/sample) using both magnetic pull-down or re-circulation capture formats. Genomic DNA was isolated and qPCR done, using a single set of primers targeting the invA gene and with two distinct TaqMan probes complementary to invA and Internal Amplification Control (IAC) for detection.

Results: Successful co-amplification of the IAC was observed in the presence of genomic DNA equivalent to $<10^3$ CFU *Salmonella*; IAC amplification was out-competed at higher target concentrations. *Salmonella* was detected, with no prior enrichment, with the pull-down assay at inoculation levels of approximately 1 CFU/9 ml; a similar detection limit was achieved with 25 ml of chicken rinse in the re-circulation assay. A gradual improvement in capture efficiency (CE) with decreasing levels of *Salmonella* contamination was observed for both assays. For example, in the pull-down assay, the CE pattern was 12% at 10^5 CFU/sample, 91.7% at 10^3 CFU/sample and 126% at 10^0 CFU/ sample.

Significance: The project demonstrates that careful design of IMS, IAC, and qPCR components can result in assay detection limits approaching those that might be anticipated in naturally contaminated foods, even without prior cultural enrichment.

P3-33 Modeling the Responses of *Lactobacillus paracasei* and *Enterobacter aerogenes* in a Gel Cassette System

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Introduction: Enterobacter aerogenes has been identified from Spanish-style green olive brines during the first fermentation phase. The last fermentation phase is characterized by an abundant growth of lactobacilli species.

Purpose: The purpose of this study was to investigate the growth rate and monitor directly the evolution of bacterial colonies of *Lactobacillus paracasei* and *Enterobacter aerogenes* when cultured individually or in combination in a gel cassette system.

Methods: The bacteria were grown in nutrient agar, with different concentrations of NaCl (0% or 3%) contained within a cassette formed between sheets of PVC film. The two microorganisms were cultured individually or in combination in gel cassettes with an initial population of ca. 10^3–10^4 CFU ml^{-1}. All cassettes were incubated at 20°C for 170 h. The primary model of Baranyi and Roberts was used to fit the growth data obtained by conventional plate counting and changes in colony area by light microscopy to derive estimates of maximum specific growth rates (lmax and Area lmax) in both cases. For curve fitting, the in-house program DMFit (Institute of Food Research, Norwich, UK) was used.

Results: Enterobacter aerogenes grew at approximately the same rate in all gel cassettes either individually or in combination with *Lactobacillus paracasei*. In contrast, *Lactobacillus paracasei* grew at a lower rate when co-cultured with *Enterobacter aerogenes*. The concentration of NaCl affected the growth rates during the first hour, but afterwards there were no significant differences. Finally, there was high correlation between lmax values estimated by conventional plate counting and area lmax values from microscopic observation in gel cassettes.

Significance: Image analysis in combination with gel cassettes could be a potential tool for rapid and convenient data collection and could also help to predict the growth rate of bacteria on food surfaces.

P3-34 Highly Specific and Sensitive Detection of *Escherichia coli* O157:H7 with Real-Time PCR

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Introduction: Escherichia coli O157:H7 contamination of food is a major public health concern because of the potential for widespread outbreaks and the severity of the enterohemorrhagic illnesses it can cause. Accurate differentiation of *E. coli* O157:H7 from *E. coli* of other serotypes is critical for identifying O157:H7-contaminated foods.

Purpose: The aim of this study was to design a sensitive, easy-to-use, and highly specific real-time PCR assay to detect *E. coli* O157:H7 while avoiding detection of other *E. coli* serotypes, including the closely related strain O55:H7.

Methods: Using a whole genome comparison approach that involved the publicly available genomes of *E. coli* O157:H7, non-O157:H7 *E. coli*, and the genome sequence of an *E. coli* O55:H7 strain generated with Applied Biosystem's SOLiD™ sequencing platform, we identified putative *E. coli* O157:H7-specific sequences that were used for real-time PCR assay designs. The specificity of the assay was determined with an inclus-ivity panel of *E. coli* O157:H7 strains and an exclusivity panel consisting of *E. coli* strains of various serotypes and closely related pathogens such as *Shigella*. The lyophilized assay, in conjunction with novel sample preparation procedures, was evaluated for sensitivity with various spiked food matrices, including ground beef.

Results: The assay was specific for *E. coli* O157:H7. Sensitivity was estimated at 10 copies using purified DNA and 1–3 CFU in 25 g of cultured ground beef within an 8 h sample-to-result workflow or after overnight enrichment for all food matrices tested.

Significance: Our *E. coli* O157:H7 real-time PCR assay is highly specific and is able to distinguish *E. coli* O157:H7 from other *E. coli* serotypes, including O55:H7, detection of which has typically been difficult to avoid due to its close phylogenetic relationship with O157:H7. The assay is also simple to use in the lyophilized format and demonstrates reliable performance, which is essential in detecting *E. coli* O157:H7 contamination in food.

P3-35 Development of a Rapid Detection Method for *Listeria* with Idaho Technology's R.A.P.I.D.® LT System in Soft Cheese and Deli Meat, and on Environmental Surfaces

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Introduction: Listeria monocytogenes has been linked to illness from the consumption of many foods. The R.A.P.I.D. LT system is optimal for rapid detection of *Listeria* in foods because of the sensitivity and specificity of PCR and rapid real-time thermocycling (30 min to result). A *Listeria* LT Food Security System (FSS) protocol, similar to the *Salmonella* LT FSS, has been designed to this end for *Listeria* species.

Purpose: To develop a rapid *Listeria* detection method that minimizes the complexity and length of enrichment, based on the principles of the *Salmonella* LT FSS (single abbreviated enrichment, 5 × 1 sample pooling, cell lysis, internal controls, and automatic analysis), for soft cheeses, deli meats, and environmental surfaces, an assay specific for *Listeria* species was developed.

Methods: Several media were tried for each matrix, and minimal enrichment times determined. 25 g portions of food were inoculated with ~1 CFU/25 g of several *Listeria* strains. Samples were enriched at 30°C in 225 ml of media and tested at different time points. Ceramic, stainless steel, and plastic surfaces (4 × 4 in.) were inoculated with low levels (~1 CFU recovered) of *Listeria*, dried, swabbed with a sponge hydrated with neutralizing broth, enriched at 30°C in 100 ml of media, and tested at different time points. All samples were evaluated individually and pooled.

Results: The optimal medium for *Listeria* detection from surfaces and deli meat is Buffered *Listeria* Enrichment Broth (BLEB), while for soft cheeses, a more selective media is required. Enrichment times vary from 24 h for environmental and cheese samples to 26 h for deli meat samples. Downstream sample processing and PCR analysis results in a final protocol time of 25 to 29 h. The system specifically detects *Listeria* species. The system is ready for method comparison and for sensitivity, and specificity evaluations.

Significance: With further evaluation, this PCR-based system is expected to provide a rapid, effective *Listeria* detection system that is faster than other methods.

P3-36 Evaluation of Idaho Technology's R.A.P.I.D.® LT *Salmonella* Food Security System in Select Foods

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Introduction: The *Salmonella* LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *Salmonella* species in food. The method take 17 h and involves: a 16 h sample enrichment, bacterial lysis to release DNA, DNA amplification polymerase chain reaction, (PCR) in the Idaho Technology R.A.P.I.D. LT instrument, internal amplification controls, and automatic result interpretation by software. Samples can be tested individually or five samples can be pooled.

Purpose: The *Salmonella* LT FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents for an AOAC evaluation study, in which *Salmonella* was spiked into cooked ham, raw chicken, and chocolate and compared to reference methods.

Methods: Several samples of each food type were prepared in 225 ml of media suggested by the reference method; 10 samples were inoculated with 1–10 CFU per 25 g, 20 samples were inoculated with 1 CFU per 25 g, and the remaining 45–25 g portion samples were left uninoculated. All were incubated for 16 h at 37°C. Samples inoculated with 1–10 CFU were pooled with negative samples post-enrichment to create 10 composite samples and tested by the *Salmonella* LT and the reference method for a side by side comparison. Samples inoculated with 1 CFU and 5 uninoculated samples were tested individually.

Results: The *Salmonella* LT FSS has the same sensitivity as reference methods for cooked ham, raw chicken and chocolate in 126 samples. The system specifically identified 120 *Salmonella* strains and did not identify 29 non-*Salmonella* species. The system is robust and reproducible as demonstrated by ruggedness, lot-to-lot studies and shelf-life studies.

Significance: This PCR-based system provides reliable detection of *Salmonella* in about 17 h as opposed to 72 h for USDA and FDA BAM methods, with fewer steps and minimal sample handling.

P3-37 Withdrawn

P3-38 Comparison of Detection Methods and Their Sensitivity in Identifying and Quantifying *Escherichia coli* O157 Isolated from Beef Carcasses and Hides

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Introduction: Bacterial loads on carcasses can differ significantly from one location on the carcass to another and from one individual carcass to another. The detection of *Escherichia coli* O157 can be difficult because of the amount of background flora and the amount of *E. coli* O157 that is found on the carcass.

Purpose: The purpose of this study was to compare the sensitivity of two enumeration methods in detecting *E. coli* O157.

Methods: Three slaughter plants in geographically distinct areas of the US were sampled over the course of a year. A total of four sampling locations on each carcass were sampled, as well as the hides. All samples were analyzed to detect and quantify the amount of *E. coli* O157 present. Samples were enumerated using a Most Probable Number (MPN) Immunomagnetic Separation (IMS) method in comparison with the ISO-GRID Hydrophobic Grid Membrane Filtration (HGMF) method. A total of 2,076 samples were analyzed.

Results: There were no positive *E. coli* O157 samples detected by the HGMF method in any sample collected in this study. The MPN method detected 25.3% positive hide samples for *E. coli* O157. The amount of pathogen quantified in these were between < 0.48–2.4 log MPN/1000 cm^2 with an average MPN of 1.5 log MPN/1000 cm^2. The percentage of positive carcass samples detected with the MPN method was 0.47% (9 total). However, the numbers in most samples were too low to be quantified with the MPN method.

Significance: Accurate measurements of both the prevalence and the total amount of *E. coli* O157 present on beef carcasses and hides is an important tool for research as well as investigative studies to determine sources and control measures for this pathogen. The IMS method we evaluated was more sensitive than the HGMF method in detecting the pathogen.

P3-39 DSC Comparative Evalution of Three Selective Media and Automated ELISA Method for Detection of *Campylobacter jejuni* in Ground Beef

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Introduction: Campylobacter jejuni has been a major cause of gastrointestinal illness throughout the world. Many selective media for detecting *Campylobacter* spp. are currently available and different countries adopt certain selective media for their official methods.

Purpose: The purpose of this study was to compare various selective media for detection of *Campylobacter* with respect to selectivity and sensitivity and to validate the VIDAS™ (bioMérieux) by comparing it to the FDA/BAM method.

Methods: Five-hundred grams of bulk sample (ground beef) was artificially contaminated with various levels of *Campylobacter* and then divided into 20 samples (25 g each). Samples were incubated in Bolton broth at 37°C (pre-enrichment for 4 h) and 42°C (enrichment for 44 h) for 48 h and then streaked onto Preston agar, CCD agar and Karmali agar. These plates were incubated under microraerobic condition at 42°C for 48 h. Presumptive identification was based on colony morphology, oxidase test, and catalase test. Subsequently, suspicious colonies were tested by API Campy for further identification. The VIDAS™ assay was performed using Bolton broth samples to compare it with the FDA/BAM method (CCD agar).

Results: When 15 CFU/500 g was inoculated, the number of positive samples from VIDAS™ (80%, 16/20) was similar from Preston agar (80%, 16/20), CCD agar (85%, 17/20) and Karmali agar (85%, 17/20). However, the numbers of plates with competing flora were significantly different. Preston agar has few competing flora (0%, 0/20), compared to Karmali agar (25%, 5/20) and CCD agar (15%, 3/20). The size of the single colony of *Campylobacter* in CCD agar was bigger than the colony on other selective media. There was no statistical difference in detection of *Campylobacter* spp. between the VIDAS™ and the FDA/BAM method.

Significance: These data show that Preston agar has better selectivity than Karmali agar and CCD agar. The CCD agar results in good growth of *Campylobacter* in comparison with Karmali agar and Preston agar. It appears that the VIDAS immunoassay system could be an alternative choice for presumptive screening of *Campylobacter* contamination in foods.

P3-40 The Use of Feline Calicivirus as an Internal Control for the Detection of Hepatitis A Virus with the PATHATRIX System

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Introduction: RNA viruses are a significant cause of foodborne illness and are associated with the consumption of a variety of foods. Because of their low infective dose and inability to replicate outside of hosts, highly sensitive detection methods are needed. Developing a common internal control for detection methods will standardize and assist with the accurate reporting of results. The technical group of the Health Canada Microbiological Methods Committee has proposed the use of feline calicivirus (FCV) for this purpose.

Purpose: We have recently developed a standardized protocol for the use of the PATHATRIX™ system (Matrix Microscience) in combination with cationic beads to detect Hepatitis A Virus (HAV) from strawberries. The application and usefulness of FCV as an internal control for the detection of HAV by this protocol was evaluated.

Methods: Fresh strawberries were spiked with various concentrations of FCV and/or HAV. The strawberries were diluted 1:10 (w/v) in buffer, and 50 µL of cationic beads were added to each sample. The samples were run through the PATHATRIX system. Viral RNA was extracted and amplified by use of RT-PCR and Real Time RT-PCR.

Results: FCV was detected constantly at the level of 10^4 PFU when added to a 25 g sample of strawberries (n = 2). Addition of 10^5 PFU of FCV to a serial dilution of HAV (100 to 10^3 PFU) did not affect the HAV detection limit. The standardized HAV extraction procedure was repeated with 10^5 PFU of FCV internal control. Detection of FCV from each sample demonstrated that the virus extraction and detection procedure was successful and that no PCR inhibitors were present.

Significance: Including an internal control in the standardized procedure for HAV detection from strawberries will enable the protocol to be used for a variety of outbreak samples with confidence. An internal control will also limit the reporting of false negative results.

P3-41 Inclusivity of Three Immunomagnetic Beads for Forty Strains of *Escherichia coli* O157

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Introduction: Rapid and selective detection of *E. coli* O157 strains has been difficult because of potential interference from background microflora. Immunomagnetic separation (IMS) methods using magnetic beads pre-coated with anti-*E. coli* O157 antibodies are able to capture and concentrate target pathogenic bacteria. The captured pathogen can then be detected by real-time PCR and isolated with standard cultural methods.

Purpose: This study was undertaken to evaluate the efficiency and selectivity of capturing target *E. coli* O157 from 40 strains in the presence of 100-fold greater levels of non-O157 *E. coli*.

Methods: Three IMS beads, PATHATRIX *E. coli* O157 IMS beads (MATRIX MicroScience), Dynabeads® anti-*E. coli* O157 beads (Invitrogen) and Dynabeads® MyOne™ anti-*E. coli* O157 beads (Invitrogen), were evaluated on BeadRetriever™ and PATHATRIX systems. Forty strains of *E. coli* O157 (food and clinical isolates) were used in this study and inoculated at ~ 2000 CFU/ml for low inoculum samples and 10 fold higher for high inoculum samples. This target organism was mixed with approximately 100 times greater background of *E. coli* 6268. Samples were plated on SMAC and TSA-YE before and after IMS. Capture efficiency and selectivity were calculated for each strain with each of the IMS beads.

Results: All 3 types of beads successfully recovered all forty strains of target organism in a background of non-O157 *E. coli* using the BeadRetriever™. In general, PATHATRIX beads captured a greater percentage of target organisms while Dynabeads® MyOne™ offered greater specificity in capturing target to non-target ratios.

Significance: Use of non-PATHATRIX beads on the PATHATRIX system was unsuccessful. However, the PATHATRIX beads did successfully recover all 40 strains of *E. coli* O157:H7 on the PATHATRIX system.

P3-42 Development of Immunochromatography Test Strip Containing Monoclonal Antibody for Rapid Detection of Ochratoxin A in White Rice

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Introduction: Ochratoxin A (OTA) is a carcinogenic mycotoxin found on agricultural products. Therefore, a rapid method is necessary to detect the toxin in foods before consumption.

Purpose: This study developed an immunochromatography test strip (ICT) and direct competitive enzyme-linked immunosorbent assay (DC-ELISA) to detect OTA.

Methods: Monoclonal antibody (MAb) from hybridoma cells of mice was purified with saturated ammonium sulfate, and MAbs were coated in microtiter plates for DC-ELISA. The purified MAbs were repurified through an affinity chromatography column and were placed on ICT pads. Cross reactivity of the two methods was evaluated to ochratoxin B, aflatoxin B1, deoxynivalenol, T-2 toxin, and zearalenone. Samples were extracted by 60% MeOH containing 1% NaCl, followed by centrifugation. The supernatant was diluted in phosphate buffered saline containing 0.05% Tween 20 to be analyzed by both methods. To determine recovery of the detection procedure, pulverized rice samples (5 g) were spiked at 5, 10, and 50 ng/g of OTA, and OTA was detected using the described method. Subsequently, developed ICT and DC-ELISA methods were used for monitoring OTA in 100 white rice samples.

Results: The MAb used in DC-ELISA showed negligible cross reactivity (< 0.1%) to Ochratoxin B, but no cross reactivity to others, while ICT had no cross reactivity with other mycotoxins. Detection range and IC50 of DC-ELISA were 0.1–10 and 0.92 ng/g, respectively. The detection limit (2.5 ng/g) of ICT was lower than that (0.06 ng/g) of DC-ELISA. The recovery with the DC-ELISA method was 80.6–111.3%. From DC-ELISA, nine samples (9%) were OTA positive at < 5 µg/kg, while all rice samples tested by ICT were OTA-negative samples.

Significance: Although ICT has lower sensitivity than DC-ELISA, the ICT is easy to use and results could be obtained rapidly (< 10 min) compared to tradi-

tional methods. The results showed that the developed ICT and DC-ELISA methods may be useful in screening of OTA in foods.

P3-43 An Independent Comparative Evaluation of the TEMPO® EB for the Enumeration of *Enterobacteriaceae* In Foods

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Introduction: TEMPO® is an automated system that enumerates *Enterobacteriaceae* present in food by combining the sample with a selective culture medium. The inoculated medium is introduced into a test card containing 48 wells across 3 different volumes. TEMPO® determines the number of *Enterobacteriaceae* present in a sample based on the Most Probable Number (MPN) method.

Purpose: The purpose of this AOAC Research Institute Independent evaluation was to compare the TEMPO® EB method for enumerating enterobacteria to the Compendium of Methods for the Microbiological Examination of Foods.

Methods: Four matrices were tested: fresh ground beef, raw cod, frozen cooked chicken and frozen green beans. Each matrix had a set of 15 samples of 3 different lots. The chicken and green beans were spiked with *Enterobacteriaceae*. Ground beef and cod were naturally contaminated. A 1:10 dilution of sample was prepared and stomached for 2 min. Two TEMPO® EB cards per sample were prepared, 1.0 ml and 0.1 ml of diluted sample was added to 3.0 and 3.9 ml of sterile distilled water in the TEMPO® vial. Cards were filled and sealed by the TEMPO® Filler and incubated for 22–27 h at 35 ± 1°C. For the reference method, samples were plated onto VRBG agar and incubated 24 h at 35 ± 1°C. Counts were obtained from typical colonies.

Results: In four matrices tested, there was no significant difference for both the mean log counts and repeatability between the TEMPO® EB and the reference method, using an independent paired *t*-test at the 95% Confidence Level.

Significance: The TEMPO® EB method has demonstrated reliability for the automated enumeration of *Enterobacteriaceae* in foods without the need for confirmation.

P3-44 A Comparison of the bioMérieux TEMPO® EC Method to the Petrifilm™ *Escherichia coli*-Coliform Count Plate Method (MFHPB-34) for the Enumeration of *Escherichia coli* from Food Products

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Introduction: In food products, microbiology quality indicator counts are mostly determined by labor-intensive tube and plate counting methods. The automated TEMPO system has been developed to replace serial dilution and plate reading with a 1/10 dilution and an automated enumeration, using a miniaturized 3 × 16 tubes MPN method.

Purpose: This study was designed to evaluate the performance of the TEMPO system for enumeration of *Escherichia coli* in naturally and artificially contaminated food products.

Methods: The TEMPO® EC method was compared to the Petrifilm™ *E. coli*-Coliform Count Plate Method described in the MFHPB-34 of Health Canada's Compendium of Analytical Methods. In this study, 25 g of product was added to 225 ml of Peptone Water and stomached. For the TEMPO® method, many different dilutions (1/10, 1/100) were added to the culture medium. Cards were filled and sealed in the automated filler and then were incubated for 24 h at 35 ± 1°C. After incubation, the cards were read in the automated reader and a result in CFU/g was generated. For the Petrifilm™ *E. coli*-Coliform Count Plate Method (MFHPB-34), decimal dilutions were transferred onto Petrifilm™ *E. coli*-Coliform Count Plates. Plates were incubated at 35 ± 1°C for 48 ± 2 h. A total of 367 samples representing 3 food categories (meat products, vegetables and dairy products) were tested and 48% of a total of 367 samples were spiked with a known *E. coli* strain

Results: Out of the entire set of data (367) and for statistical purpose, we only take into consideration the results that can be converted into $\log_{10}$ (146). When analyzing the 146 results within range for both methods, the results show a percentage of agreement (less than one log between both methods) of 97.9%, which represents 3 samples (2 vegetables and 1 dairy product). Regression analysis of log counts demonstrate good correlation between the new method and the reference method.

Significance: In terms of overall accuracy, the TEMPO® EC method is equivalent to the Petrifilm™ *E. coli*-Coliform Count Plate Method (MFHPB-34) for the enumeration of *E. coli* from food products.

P3-45 Performances of the TEMPO® STA Method in Comparison with Conventional Plate Count Method for Enumeration of *Staphylococcus aureus* in Food Samples

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Introduction: Enumeration of coagulase-positive staphylococci, mainly *S. aureus,* in food samples remains critical to monitor manufacturing processes and release finished goods. The food industry is constantly on the lookout for microbiological methods which are less time-consuming and labor intensive than the traditional ones.

Purpose: The goal of the study was to compare the new automated TEMPO STA method with the conventional plate count method for enumeration of *S. aureus* in food matrices.

Methods: A total of 114 collection strains belonging to staphylococcal species and other genera and species were first tested on the TEMPO STA medium. Then, the TEMPO STA medium was evaluated on 99 fresh food samples compared to the conventional Baird Parker plate count (ISO 6888-2 reference method) and one alternative method for comparison purposes. Samples included raw to processed products. After inoculation, the TEMPO STA cards were incubated for 24 h at 37°C. Data obtained on the 3 methods were compared using a combination of statistical tests.

Results: All *S. aureus* collection strains were detected on the medium. Regarding the food samples, 50 fresh matrices appeared contaminated by *S. aureus* and gave a positive result on at least one of the three methods. Data obtained with the medium were in agreement with those obtained with the alternative and the reference method. However, in case of high background flora, the test was the only method able to provide a precise count by an efficient growth inhibition of non *S. aureus* cells on the medium.

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Significance: The new test offers a rapid and automated method able to give a result for enumeration of *S. aureus* in 24 h without any confirmation test.

P3-46 Evaluation of a New Automated Lactic Acid Bacteria Method for Enumeration in Food Products Using the TEMPO® System

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Introduction: Whereas lactic acid bacteria (LAB) are considered beneficial in the manufacture of fermented products, they can also act as spoilage organisms, known to shorten product shelf life and degrade product quality. The traditional methods for enumerating LAB in food products involve time-consuming plating techniques. TEMPO LAB is a method for lactic acid bacteria enumeration based on the familiar format of the MPN procedure.

Purpose: In this study, we compared the new LAB enumeration method to the Compendium method for the microbiological examination of foods: MRS agar incubated at 35°C anaerobically for 72 ± 3 h.

Methods: In this study, more than 300 naturally contaminated products from three different sites were tested from different food categories such as raw and cooked meat and poultry products, fish and seafood products, vegetables, dairy products, ready-to-eat (RTE) and environmental samples. TEMPO LAB uses a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the MPN. This test provides results within 40–48 h of aerobic incubation at 35°C compared to 72 ± 3 h for the reference method with anaerobic incubation conditions. A combination of regression analyses, difference $\log_{10}$ distributions and *t*-tests at the 5% level were used to analyze the data and compare performances.

Results: This method showed performance similar to that of the reference, with an agreement level greater than 95% on the whole data. Regression analysis gave coefficients of variation close to 0.9, with a slight negative bias due to better selectivity than that of the Reference Method.

Significance: The new method gives quantitative results similar to those of the Reference Method and offers an alternative rapid automated enumeration method for food laboratories.

P3-47 Evaluation of a New Method for the Enumeration of *Enterobacteriaceae* in Foods

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Introduction: The TEMPO® EB (*Enterobacteriaceae)* method was developed for the automated enumeration of *Enterobacteriaceae* in foods. This method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number.

Purpose: As part of the AOAC Research Institute validation process, the new method was compared to the Compendium of Methods for the Microbiological Examination of Foods for all foods.

Methods: Eighteen naturally and artificially contaminated foods were tested, including meat, poultry, egg products, dairy, fish and seafood, and vegetables. For each food, three lots and five replicates of each lot were tested, for a total of 270 samples. A 1:10 dilution of each sample was prepared and stomached for 2 min. For each diluted and stomached sample, 1.0 ml of diluted food sample was added to a TEMPO medium vial that had been reconstituted with 3.0 ml of sterile distilled water. The inoculated medium in the vial was then transferred and sealed into the EB card by the automated filler. The inoculated EB cards were incubated for 22–27 h at 35 ± 1°C. Cards were read using the automated reader. Standard method testing was performed as detailed in the Compendium.

Results: For the majority of samples tested, there was no significant difference for both the mean log counts and repeatability between the TEMPO EB method and the standard method, using a paired *t*-test and *f*-test at the 5% level.

Significance: The new method provides an automated, accurate method for the enumeration of *Enterobacteriaceae* in foods.

P3-48 Evaluation of a New Method for the Enumeration of Coliforms in Foods

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Introduction: The TEMPO® CC (Coliform Count) method was developed for the automated enumeration of coliforms in foods. This method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number.

Purpose: As part of the AOAC Research Institute validation process, the new method was compared to the Standard Methods for the Examination of Dairy Products (SMEDP) for dairy products and the FDA Bacteriological Analytical Manual (BAM) for all other foods.

Methods: Eighteen naturally and artificially contaminated foods were tested, including meat, poultry, egg products, dairy, fish and seafood, vegetables, and animal feeds. For each matrix, three lots and five replicates of each lot were tested, for a total of 270 samples. A 1:10 dilution of each sample was prepared and stomached for 2 min. For each diluted and stomached sample, 1.0 ml of diluted food sample was added to a medium vial that had been reconstituted with 3.0 ml of sterile distilled water. The inoculated medium in the vial was then transferred and sealed into the CC card by the automated filler. The inoculated cards were incubated for 22–27 h at 35 ± 1°C. Additionally, dairy products were also tested on a second card that was incubated at 32 ± 1°C. Cards were read using the automated reader. Standard method testing was performed as detailed in the SMEDP for dairy products and in the BAM for all other foods

Results: For the majority of samples tested, there was no significant difference for both the mean log counts and repeatability between the new method and the standard method, using a paired *t*-test and *f*-test at the 5% level.

Significance: The new method provides an automated, accurate method for the enumeration of coliforms in foods.

P3-49 DSC Binding Characterization of *Listeria* Adhesion Protein from Different *Listeria* Species to Its Eukaryotic Receptor Hsp60 with a Surface Plasmon Resonance Biosensor

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Introduction: In genus *Listeria*, *L. monocytogenes* and *L. ivanovii* are pathogenic while others are non-pathogenic. *Listeria* adhesion protein (LAP), though present in pathogenic and non pathogenic *Listeria* spp., is considered a virulence factor, required for the adhesion of *L. monocytogenes* to the intestinal epithelial cells. A chaperone protein, Hsp60, acts as host cell receptor for LAP.

Purpose: Here, we investigated the binding kinetics of LAP from different *Listeria* spp. to Hsp60 to determine the role of LAP in pathogenesis.

Methods: LAP from different *Listeria* spp. was cloned, expressed in *E. coli* and purified with Ni affinity chromatography. Graded concentrations of recombinant LAP from *L. monocytogenes* ($rLAP_{mono}$), *L. innocua* ($rLAP_{inn}$), *L. ivanovii* ($rLAP_{iva}$) and *L. welshimeri* ($rLAP_{wel}$) were added to the cuvette immobilized with Hsp60 and LAP binding was monitored in a SPR sensor. The association (ka) and dissociation rate constants (kd) and dissociation equilibrium constant (K_D) of LAP-Hsp60, interaction was calculated, using Graphpad.

Results: A concentration dependent increase in response was observed when rLAP from different *Listeria* spp. was added to Hsp60. The ka of $rLAP_{iva}$, $rLAP_{wel}$ and $rLAP_{inn}$ were calculated to be 2.51×10^7 $M^{-1}s^{-1}$, 1.48×10^9 $M^{-1}s^{-1}$and 2.23×10^8 $M^{-1}s^{-1}$, respectively, which was similar to that of $rLAP_{mono}$. Also the K_D values for $rLAP_{iva}$ and $rLAP_{inn}$ were determined to be 2.17×10^{-5} M and 4.56×10^{-7} M, respectively. The binding kinetics values obtained were not significantly different from the values for the $rLAP_{mono}$, reported previously.

Significance: Though the binding affinity of LAP from different *Listeria* spp. to its receptor Hsp60 is similar, differential display of LAP on the surface is possibly the determining factor for LAP to serve as an adhesion factor in pathogenic *Listeria*. Also, the results indicated that Hsp60 could potentially be used as a capture molecule for the detection of *Listeria* on various assay platforms.

P3-50 DSC Chromatographical Method for Monitoring of Patulin in Fruit Juices Produced in South Korea

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Introduction: Patulin has been known to be a carcinogenic mycotoxin produced by species of *Penicillium* and *Aspergillus*, and it has been found in many fruit juices. Therefore, effective methods need to be established for detection of patulin in fruit juices.

Purpose: This study established a detection method using high performance liquid chromatography (HPLC) equipped with a photodiode array detector to measure concentrations of patulin in fruit juices.

Methods: Samples were extracted by solutions in the following order: ethyl acetate, 1.5% sodium carbonate, anhydrous sodium sulphate, and ethyl acetate. The organic solvents were then evaporated under nitrogen flow, and the dry residue was reconstituted in dH_2O (pH 7.4), to be analyzed by HPLC (mobile phase: 10% acetonitrile; flow rate: 1.0 ml/min). In order to validate this procedure, linearity (R2) of standard curves prepared at 0–500 ng/ml of standard solutions was determined. Moreover, apple, orange and pear juices were spiked with patulin at 20–50 ng/ml, 50 ng/ml and 5–10 ng/ml, and samples were analyzed by HPLC to determine precision and accuracy, specificity, and detection and quantification limit, respectively. Subsequently, the validated detection method was used for monitoring of patulin in 120 samples of fruit juices (apple, grape, orange, pear, tangerine; 24 samples/juice).

Results: In validation, accuracy (recovery: 96.03–117.77%) and precision (standard deviation: 3.35–9.52) of the method were acceptable. Detection (10 ng/ml) and quantification limit (15 ng/ml) were determined. R2 of the standard curve was 0.99853. Of 120 tested samples, 10 samples (apple: 3, grape: 5, orange juice: 2) were patulin positive at levels of 2.8–30.9 ng/g, which were above the tolerable daily intake (0.5 ng/kg).

Significance: These results indicate that the optimized, validated method using HPLC was suitable for monitoring of patulin in fruit juices. Furthermore, since contamination level of patulin was high in fruit juices, continuous monitoring for patulin and risk assessment should be conducted.

P3-51 Advantages of Bacteriophage in Conventional Selective Agars for the Isolation of *Salmonella*

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Introduction: Conventional isolation of *Salmonella* is accomplished by cultural methods using multiple selective agents. The addition of bacteriophage to competing organisms, as an additional selective agent to commercial plating media, complements and enhances specificity of conventional methods for isolation of *Salmonella* species.

Purpose: This study demonstrates the effectiveness of bacteriophage blends to reduce growth of competing bacteria in commercial plating media, thus allowing easier identification of characteristic *Salmonella* colonies in high burden samples.

Methods: Poultry drag swabs were compared by conventional methods and by those using bacteriophage as a additional selective agent against competing bacteria. One half of the swab was added to 50 ml of SDI's RapidChek SELECT *Salmonella* primary media and incubated at 42°C for 16 h, followed by a secondary enrichment at 42°C for 20 h. The other half of the swab was added to 50 ml of tetrationate broth as a delayed secondary enrichment method, as specified in the National Poultry Improvement Plan (NPIP) method. All samples were struck onto conventional BGN and XLT4 agars as well as onto BGN and XLT4 agars supplemented with bacteriophage. Plates were examined for the presence of *Salmonella* based on morphology and were serologically and biochemically confirmed.

Results: Selective plates with bacteriophage had significantly reduced growth of non-*Salmonella* colonies when compared to conventional selective plates. This facilitated isolation of typical *Salmonella* colonies, without the need for additional incubation time.

Significance: Addition of bacteriophage to conventional plating media increases the sensitivity and specificity of *Salmonella* isolation and confirmation. This is of particular advantage with high burden samples, such as poultry drag samples. The addition of bacteriophage reduces the number of non-*Salmonella* colonies tested, thus improving the efficiency of laboratory work. The risk of releasing product contaminated with *Salmonella* is reduced with the increased efficiency of identification procedures.

P3-52 DSC Impact of Growth Phase, Chemicals and Food Matrices on Bacterial Differentiation Using FTIR Spectroscopy

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Poster

Introduction: Fourier Transform Infrared (FTIR) spectroscopy provides a rapid, highly selective and reproducible means for the chemically based discrimination of intact microbial cells, which makes the method valuable for large-scale screening of foods.

Purpose: The goals of the present study were to assess the effect of chemical interferents, such as food matrices, different sanitizing compounds and growth media, on the ability of the method to accurately identify and classify *L. innocua*, *L. welshimeri*, *E. coli*, *S. choleraasuis*, *S. subterranean*, *E. sakazakii*, and *E. aerogenes*. Moreover, use of FTIR spectroscopy for discrimination of *L. innocua* and *L. welshimeri* of different genotypes and the effect of growth phase on identification accuracy of *L. innocua* and *L. welshimeri* were tested.

Methods: FTIR spectra were collected, using two different sample presentation techniques – transmission and attenuated total reflection (ATR) – and then analyzed using multivariate discriminant analysis based on the first derivative of the FTIR spectra with the unknown spectra assigned to the species group with the shortest Mahalanobis distance.

Results: The results of the study demonstrated 100% correct identification and differentiation of all bacterial strains used in this study in the presence of chemical interferents or food matrices, and a greater than 99% identification rate in the presence of media matrices. FTIR spectroscopy proved to be 100% accurate when differentiating between genotypes of *L. innocua* and *L. welshimeri*, with the classification accuracy unaffected by the growth stage.

Significance: These results document that FTIR spectroscopy can reliably and accurately identify and differentiate foodborne pathogens in foods and environmental samples without interference from food matrices, chemical sanitizers or growth media.

P3-53 DSC Insight into Asian and Hispanic Restaurant Managers' Needs for Safe Food Handling

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Introduction: The rise in ethnic food popularity in the US has brought increased risks: Between 1990 and 2000, foodborne illness outbreaks associated with ethnic foods rose from 3% to 10%.

Purpose: This study identifies the reasons behind the rising incidence of foodborne illnesses associated with ethnic foods and corresponding ways to improve current food safety educational programs.

Methods: One on one interviews were conducted with managers of 41 Asian (n = 21) and Mexican (n = 20) food vendors in Northern California; the interviews lasted for 20–40 min. Questions addressed basic food safety procedures, challenges in meeting food safety requirements, and suggestions on how to improve food safety education programs.

Results: Despite assurance on confidentiality, interviewer association with an educational institution rather than a regulatory body, and the offering of an incentive, volunteer rate was only 20%, with 209 restaurants contacted to obtain 41 volunteers. Restaurant managers discussed the difficulty of maintaining proper food temperatures, the need for classes that used examples of food/procedures common to their food offerings, the need for instructional videos and posters for employees, the difficulty of having only one individual responsible for disseminating safety information to employees, the element of unpredictability in running a restaurant, the need for inexpensive and in-house staff-training programs, and the high employment-turnover rate in the restaurant industry. Some interviewees candidly admitted that they did not always follow recommended practices because they felt it was inconvenient to do so.

Significance: Food safety educators should consider tailoring educational programs to the food examples and language needs of the ethnic community. Educators should also develop methods to clearly demonstrate the consequences of not following critical food handling procedures. The knowledge gained in this study could help food safety professionals increase the effectiveness of educational programs directed toward Asian and Hispanic restaurants.

P3-54 Promoting Science-Based Home Food Preservation Learning for Adult Consumers through the Use of Online Educational Tools

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Introduction: Home food preservation is a significant activity in the US. Disseminating safe procedures reduces the risks of food spoilage and disease and embraces the emerging movement towards preserving a homegrown food supply. Forty million Americans use the Internet as their main source of science information. Promoting online learning ensures accessibility and visibility of science-based educational and reference materials.

Purpose: The objective of this project was to construct, maintain and evaluate a web site containing USDA and Extension home food preservation recommendations.

Methods: The web site (http://www.homefood-preservation.com) was launched in April 2002 and was followed by a criterion-based initial site design analysis. A survey group of 37 educators and consumers and a pop-up survey for web site visitors provided preliminary user feedback. Ongoing evaluation includes visitor feedback and multi-institutional, directed surveys.

Results: The web site has 886 HTML pages, 158 PDFs, 31 PowerPoint files, 7 multimedia tools, a search function, and an 'Information Request' form. During 2005–2007, an annual average of 840,000 visits were recorded, and 1500 US and international visitors were assisted by e-mail. Eighty-six percent of the "initial survey" respondents indicated the website structure 'well categorized and easy to follow'. Eighty-five percent found the search features 'very useful'. One-hundred percent indicated that the web site would be a positive influence on consumers. The "pop-up survey" determined that 92% of respondents (n = 129) found the web site information helpful and planned to adopt it. The web site has been cited as a resource in print publications and online articles, indicating an increased Internet presence.

Significance: The web site is successful in meeting objectives of making food preservation recommendations available to first-time and skilled food preservers, food business professionals, educators, researchers, students and the media. It fosters consumers' personal responsibility for food preservation safety, indicated by reported changes in practices and awareness. The largest number of visitors currently 'Direct Request' the site as the result of popularity and repeated use.

P3-55 When Do Dieticians and Nurses Provide Food Safety Information to High Risk Populations?

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Introduction: At-risk populations are vulnerable to foodborne illnesses and it is critical that they have credible sources of information to protect against infection. At-risk clients consider their health professionals to be credible sources of information. Information relative to the accuracy of health professionals' food safety knowledge, the messages they provide and the educational methods they use with their at-risk clients is limited.

Purpose: Dieticians and nurses are health professionals in close communication with at-risk clients. Understanding dieticians' and nurses' motivation for providing food safety education, and knowing the content and preferred delivery methods, are necessary to improve food safety education. The professionals' knowledge may ultimately affect the well-being of the at-risk consumer.

Methods: A web-based survey was developed and marketed to dieticians and nurses working with at-risk groups (i.e., pregnant women, the elderly and immune comprised patients). Questions accessed what, why, when and to whom food safety information is provided by the health professional.

Results: A total of 61 nurses and 73 dieticians responded to the survey. Of the 59% of nurses and 84% of dieticians who provided food safety information to at-risk groups, only 19% of the dieticians and 18% of the nurses thought it was their responsibility to do so. The most prevalent reason for providing information was having a client indicate a risky food behavior practice (dieticians, 46% and nurses, 25%). The two protective practices suggested most often were handwashing (dieticians 72% and nurses 97%) and avoiding unsafe food sources (dieticians 76% and nurses 86%). Informal verbal education was the most common educational method used by dieticians (80%) and nurses (93%).

Significance: Since at-risk clients rely on health professionals to provide accurate and appropriately delivered food safety education, the information learned in this study will inform the development of educational programs for health professionals as well as at-risk consumers.

P3-56 Food Safety Practices and Educational Needs of Dietary Managers in Nursing Care Facilities

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Introduction: Persons in nursing and long-term care facilities are typically susceptible to listeriosis due to compromised immune systems. Dietary managers of such facilities can play a critical role in minimizing this risk through the foods they serve, the protocols they allow and the training they provide food service staff and residents.

Purpose: This study was designed to better understand what dietary managers who serve high risk patients know about *Listeria monocytogenes*, special precautions they take to enhance the safety of the food they serve, and their food safety training needs.

Methods: Members of the national Dietary Managers Association were recruited through listservs and web newsletters to take a web-based survey.

Results: Most of the 122 dietary managers who responded worked for long-term or skilled-care facilities. The majority (65%) said they had heard little or nothing about *Listeria*. While they had received training on food safety, only 17% indicated receiving specific training on *Listeria*. These dietary managers commonly served luncheon meats, hot dogs and prepared salads to their high risk patients and 28% served soft-cooked eggs. Six to 12% also served meat pates, soft cheeses like queso fresco, and raw or smoked fish. Participants reported storing opened packages of luncheon and deli meats for a wide range of times. While most reported having standard protocols for cleaning and sanitizing meat/cheese slicers, kitchen counters and refrigerated spaces, fewer had standard protocols for cleaning kitchen disposals, floor drains and floor sinks. Participants indicated strong interest in receiving training on minimizing the risk of listeriosis among high risk patients and were most interested in receiving this training via the web or CD-ROM.

Significance: The information gained will help guide the development of web-based training for dietary managers designed to help minimize the risk of *L. monocytogenes* in establishments that serve patients at high risk of foodborne illness.

P3-57 Preparation and Storage of Reconstituted Powdered Milk Formula: Caregiver Perspectives

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Introduction: Methods used to prepare and store reconstituted powdered milk formula have important microbiological implications for safety. Recommended procedures in the home and healthcare settings may be achieved by equipping parents and caregivers with adequate/correct knowledge, positive attitudes and motivation to implement desired behaviors that can minimize microbial risks.

Purpose: This study aims to understand parent and caregiver beliefs, attitudes, practices and information provision relating to infant feeding with powdered milk formula.

Methods: Sixteen focus groups were conducted in seven locations across the UK and included homogenous groups of parents, day-nursery-nurses, hospital-nurses and health-visitors. Respondents were recruited according to employment responsibilities, frequency of formula preparation and demographics. Each group discussion followed a structured guide and included evaluation of educational materials.

Results: The majority of respondents were unaware of specific microbiological risks associated with formula and considered current practices to be 'safe'. Some parents reported storage of reconstituted formula at ambient temperature when away from the home; others reported to 'take water and powder separately' and reconstitute formula immediately before use. Common sources of formula information for parents included family, friends, midwives and health-visitors. A frequent barrier to obtaining information from professional sources was an attitude of being 'dead against formula'. Nurses and health-visitors reported having limited information to distribute regarding formula feeding and cited 'lack of

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time' and conformity to the WHO Baby Friendly Initiative as reasons for not providing formula based advice or bottle demonstrations. Further discrepancies between caregiver groups will be discussed, particularly regarding information provision.

Significance: Findings from this study will help in the development of effective, targeted information sources that address microbial risks of preparation and storage of powdered formula milk.

P3-58 DSC Characterization of Food Safety Knowledge and Behaviors of Adolescents

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Introduction: Educational interventions can improve food safety knowledge and behaviors if they are aligned with specific needs of target groups. Establishing a baseline for food safety knowledge in adolescents is important because it is understudied in this group, and research shows adolescence is an ideal time to establish life-long behaviors.

Purpose: The objectives of this study were to: (1) develop a statistical sampling method to measure food safety knowledge and behaviors of 7th grade students in East Tennessee; (2) identify gaps in food safety knowledge and behaviors; and (3) determine the impact of geographic location, socioeconomic status, race, and gender.

Methods: A 40-item survey assessing food safety knowledge and behaviors was administered to 232 students in 13 schools chosen, using a weighted, stratified random sample. A hierarchical model was used to obtain least squares means at the school and student levels.

Results: Results showed that 63% knew the importance of hand-washing, but only 50% reported 'always' washing their hands before eating or preparing food; 50% reported 'always' following temperature directions, but 85% did not know how to determine if a hamburger was cooked properly, and 74% did not know how to safely thaw meat. No statistical difference was found in food safety knowledge for all variables except race, where Asian/Pacific students scored lower ($P = 0.0005$). Males ($P = 0.0133$) and Asian/Pacific students ($P = 0.0033$) reported riskier food handling behaviors.

Significance: Hand-washing and use of proper temperatures, as well as differences in behavior within gender and some ethnic groups, should be focal points in adolescent food safety education. Our results suggest that some differences in knowledge and behaviors are less pronounced in adolescents than those found in similar studies with adults. With limited food handling experience and less impact from demographic factors, dissemination of knowledge and development of safe behaviors through adolescent education may prove successful in improving consumer food safety.

P3-59 Food Safety Labels and Education for Meals-on-Wheels Participants

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Introduction: Meals-on-Wheels (MOW) recipients may be more at-risk for foodborne illness than healthy elderly adults. Delivered meals usually do not have food handling/safety labels which provide information on proper storage and reuse of uneaten foods. Limited food safety education is provided for MOW recipients.

Purpose: To develop user-friendly food safety labels for home-delivered meals and food safety education materials for MOW recipients.

Methods: Five focus groups were conducted with seniors who ate at centers where meals were prepared for MOW recipients. Six food safety labels and five food safety handouts were developed for the seniors and cooks to analyze during the focus group sessions. After data analysis, one label was developed and the food safety handouts were revised for testing with MOW recipients. One week after the label was used and the handouts delivered with meals, an interview was conducted with MOW recipients to determine their effectiveness.

Results: Forty-three seniors and nine cooks participated in the focus groups. Responses to sample labels included: keep it simple, use large black print, concerns about how to date the label, and concerns about the reheating statement. Comments about the educational materials included: liked large print, liked colored picture, and keep statements simple. Of the 47 MOW recipients interviewed after one week, 94% stated that they read the label on their delivered meals, whereas 91% read the educational materials. Only 19% stated the correct refrigeration temperature (information given on label) but everyone felt that their refrigerator worked properly. Only 72% knew how long to properly store leftovers (information given in the educational materials).

Significance: Our study demonstrated that a food safety label for MOW home-delivered meals was needed to remind participants how to store uneaten foods safely. Educational materials are needed occasionally as a reminder of safe food handling practices.

P3-60 Successful Listeriosis Prevention Continuing Education Seminars for Health Professionals Working with Pregnant Women

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Introduction: An estimated 2,500 cases of listeriosis occur annually in the United States, primarily affecting the fetuses of pregnant women and resulting in a 20–30% death rate. Health care professionals are key messengers in providing food safety information to pregnant women.

Purpose: Based on results of a previous study assessing food safety information needs of health care professionals, these researchers developed and conducted both live and web-based seminars on listeriosis prevention for WIC and other prenatal program staff who counsel pregnant women. One continuing education credit was provided through the Colorado Dietetic Association.

Methods: Postings to the Public Health Nutrition listserv and USDA WIC-WORKS website helped recruit participants to enroll in one of three webinars entitled: "Promoting Safe Food Practices during Pregnancy, with Emphasis on *Listeria*." Pre and post surveys were conducted via anonymous live polling using the BREEZE web conferencing system through Iowa State University. Additionally, Colorado Extension Agents conducted live trainings to health department staff within their local communities.

Results: A total of 385 health professionals from 45 states and one territory participated in the three webinars and the five live trainings (264 WIC dieticians/

nutritionists, 55 nurses and 65 WIC program staff). Eighty-seven percent of participants reported that they currently provide food safety information to their pregnant clients. Knowledge scores improved by up to 41% from pre to post test, and most (64%) participants indicated they planned to make changes in the food safety recommendations they give to pregnant women as a result of the training.

Significance: While live trainings are desirable when possible, these researchers conclude that the webinar format is a highly effective continuing education option for WIC staff, nurses and other professionals. A recorded webinar has since been made available for download to promote further outreach efforts. These efforts will help minimize risk of listeriosis among pregnant women.

P3-61 An Evaluation of the Technical Development Programs and Training Needs of Food Manufacturers

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Introduction: As part of a 'due diligence' defense, UK food companies are required to demonstrate food safety training "commensurate with job activities". This definition allows for a variability in the interpretation and delivery of training strategies and programs.

Purpose: The purpose of this research was to establish an understanding of the strategy, delivery and content of existing training programs and future training needs within the food processing sector.

Methods: The research was conducted using a semi-structured interview with 15 food manufacturing companies. The companies varied in size from SME to major international food processors.

Results: One-hundred percent of companies indicated that training was "important or very important" to their company; however the largest spent on training was only 1% of company turnover (500,000 dollars).

The most frequently cited barrier to training was cost (80% of respondents). Sixty-five percent of companies indicated that they had suffered financial losses in productivity and quality inefficiencies as a result of gaps in training delivery. When questioned on specific technical subjects, 71% of categories were rated as poor or very poor for external provision of such training. The preferred method of training delivery was a maximum of ½ day blocks. The reason for this will be discussed. Fifty-three percent of companies preferred exam to competency based assessments of training. When questioned about their company's training program in relation to other similar sized companies, 93% believed that their programs were better or on a par.

The external qualifications gained by the research group showed that < 3.5% related to a managerial focused qualification, with the remainder gaining basic vocational qualifications.

Further data developed during the research project, including the group's understanding of training availability, government based sector specific training bodies and perceptions of the quality of external training providers, will be discussed.

Significance: The university plays an integral part in the strategic development of food strategy and policy in Wales and the results of this research will be used to advise the Welsh Assembly Government on future funding in food related training.

P3-62 A Pilot Study of the Conference for Food Protection's Proposed Model for Training and Standardizing Food Safety Inspection Officers in Retail Food Regulatory Agencies

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Introduction: The effectiveness of retail food regulatory programs depends to a great extent on the ability to employ Food Safety Inspection Officers (FSIOs) who possess the knowledge and skills needed to effectively conduct food establishment inspections and communicate their findings to the person in charge of the facility. Most regulatory agencies provide training for newly hired or assigned FSIOs. However, the content and scope of this training varies greatly from jurisdiction to jurisdiction. What is needed is a nationally recognized training and standardization process for FSIOs that can be used to enhance the effectiveness of food safety inspections and increase uniformity among regulatory professionals in the assessment of food safety practices in the retail food industry.

Purpose: The purpose of this study was to evaluate a five-step training and assessment process created by the Conference for Food Protection to serve as a national model for preparing FSIOs to conduct inspections of restaurants, grocery stores, and institutional foodservice establishments. The training process included coursework available through the Food and Drug Administration's Office of Regulatory Affairs' online university (ORAU), or equivalent; a minimum of 25 joint field-training inspections; and a minimum of 25 independent inspections. The 25 joint field inspections included both "demonstration" (trainer-led) and "training" (trainee-led) inspections for all categories of food establishments available within the jurisdiction. As part of the joint field-training inspections, the jurisdiction's trainer or designated staff person conducted an Assessment of Training Needs (ATN) to determine the FSIO's readiness to conduct independent inspections.

Methods: A multi-step training and assessment process was implemented by 29 retail food regulatory programs between June 2006 and July 2007. The study jurisdictions ranged in size from less than 25,000 to more than 500,000 and represented all levels of government including State (9), County (11), District (2), City (6), and Tribal (1). One hundred and thirty-two FSIOs participated in the study and a total of 110 FSIOs completed all of the steps in the training and assessment process.

Results: More than 96% of the study participants agreed that the training and assessment process was a valuable use of their agency's resources. Almost all jurisdictions (89.6%) agreed the 25 performance elements contained in the Field Training Worksheet tool addressed the knowledge and skills a FSIO needs to effectively conduct independent retail food establishment inspections. Nineteen of the pilot jurisdictions (66%) agreed that a minimum of 25 joint field-training inspections was adequate to prepare FSIOs to conduct independent inspections. However, many of the respondents believed the number of required joint inspections should be variable based on an individual FSIO's prior experience, skills, capability and affinity for learning new tasks, or accomplishment of certain skills.

Based on the input gained from this study, it is evident that the training and assessment process and forms are valuable tools for retail food regulatory

programs. When used properly this process will enable agencies to standardize their training programs for FSIOs and provide the knowledge and skills FSIOs need to effectively conduct independent inspections of retail food operations.

Significance: By implementing a nationally recognized training, assessment and standardization process for Food Safety Inspection Officers (FSIO), retail food regulatory programs will be able to enhance the effectiveness of food safety inspections and increase uniformity among regulatory professionals in the assessment of food safety practices in the retail food establishments.

P3-63 DSC Analyzing the Social Costs of Food Safety Failures

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Introduction: Food safety failures have dramatic effects on public health and often trigger a broad range of policy interventions. Economic research has evaluated food safety policy, exploring the costs and benefits to consumers and/or firms. However, this literature includes few studies that consider impacts to both groups simultaneously.

Purpose: In pursuing this goal, this study estimates the social costs and benefits of food policy by considering the effectiveness of foodborne illness controls. A key aspect of this analysis is the time variant nature of costs and benefits. By accounting for dynamic characteristics of the relationship between firms and consumers, social costs from food safety failures can be more accurately estimated.

Methods: Considering the identification of how each side's costs are linked, this paper discusses time varying costs, recognizing the importance of the length of time people are sick from contaminated foods, how long firms take to recall products and the period in between recalls. The empirical study builds on Roe (2004) and assesses two types of policy. One attempts to reduce the marginal social cost of food safety failures; the other attempts to strengthen risk assessments, accelerating recalls and/or enhancing recovery rates.

Results: Exploring this data using a probit specification, preliminary results suggest that the probability of having a recall in a certain state is affected by the lag of outbreaks rather than current outbreak cases in the state. Costs associated with recalls and outbreaks are incurred over different time periods. Interestingly, firm burdens do not appear to be related to how long people have been sick.

Significance: Therefore, an appropriate policy tool for food safety failures may be focused on creating a more rapid recall identification system employing more aggressive testing and risk communication practices.

P3-64 DSC A Socio-Psychological Model Applied to the Implementation of Food Safety Management Systems

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Introduction: Small and medium sized food businesses have been slow to adopt food safety management systems (FSMSs) such as Hazard Analysis Critical Control Point (HACCP). Socio-psychological models of barriers to and facilitators of the implementation of FSMSs in food processing facilities have not been validated and may be based on models without explanatory or predictive power. Furthermore, most models do not appear relevant at the production level. The theory of planned behavior has been shown to have explanatory and predictive power in some settings: it has shown perceived control over one's own behavior, one's attitude and the influence of others to be antecedents of behavioral intention that leads to behavior.

Purpose: The objectives of this study were to identify factors that influence production workers in the implementation of FSMSs in small and medium meat processing facilities and determine how these factors are applicable to the theory of planned behavior.

Methods: A qualitative approach was used: thirteen in-depth interviews at meat plants and two focus groups with representatives of government and industry agencies generated 219 pages of verbatim transcripts. Transcripts were analyzed using NVIVO software. Triangulation increased the dependability of the data.

Results: Themes arising from the data include facilities and equipment, approach to integrating FSMSs, management actions, training, and supervision and feedback. It appears that most factors influencing production workers relate to their perceived behavioral control over food safety related behaviors and tasks.

Significance: Further research to confirm the factors having the strongest influence on production workers following food safety practices in meat plants, and validation of the theory, would make it possible to target interventions to improve the implementation of FSMSs in the meat industry.

P3-65 Determining the Level of Compliance with Legal Traceabilty Requirements

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Introduction: Recently introduced EU Regulations made Traceability a requirement and although larger companies have operated systems for some time, many small and less developed businesses have never operated this level of control. The aim of the legislation is to allow traceability from the primary producer to the final consumer, requiring each producer in the chain to demonstrate knowledge of their direct suppliers and immediate customers. If every producer demonstrates this, traceability throughout the whole food chain is achieved.

Purpose: The aim of this research was to determine both the level of compliance and suitability of the requirement.

Methods: A self assessment/audit questionnaire, covering knowledge of the regulation, traceability systems and implementation, was devised and posted to 250 businesses in various food sectors. Each business was invited to undergo an additional audit against a specially developed Traceability Audit Questionnaire. This second phase questionnaire included both mandatory requirements and industry accepted good practice. The audits were undertaken by an experienced auditor with detailed knowledge of food safety and food law.

Results: Forty-eight questionnaires were returned, and reported practices indicated a high level of knowledge and compliance, although 20% of respondents were unaware of this legislation. The remaining respondents reported total compliance, although 10% indicated that they were unaware of all the requirements of a traceability system. Fifty percent of the initial respondents were audited for the second phase, with only

63% of these approaching the legal minima and only 2 companies able to demonstrate sufficiently detailed traceability to the level required by certified food safety standards, including BRC and ISO22000.

Significance: These findings indicate that although regulations may be introduced, there is a lack of understanding of their requirements and how to achieve compliance. The well documented shortage of both EHOs and Food Technologists is clearly impacting on the efficacy of control and potentially on consumer safety.

P3-66 DSC Risk Communication and the Lessons Learned from the 2007 Melamine Associated Outbreak: Potential Food Safety Benefits

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Introduction: In March 2007, an outbreak of pet food-associated nephrotoxic renal failure occurred in North America, affecting large numbers of dogs and cats. In the ensuing months, a series of investigations revealed the presence of melamine and cyanuric acid in various brands of recalled pet foods. During the crisis, various institutions were charged with communicating with the public to reduce and contain harm.

Purpose: The purpose of the study was to assess the application of the 10 best practices of crisis and risk communication by institutions (United States Food and Drug Administration (FDA), pet food manufacturers and distributors) during the 2007 melamine associated pet food outbreak.

Methods: Risk messages and responses to the public from the FDA and 12 major pet food manufacturers and distributors were examined and assessed against the 10 best practices of crisis and risk communication. The messages were drawn from the FDA websites, pet food manufacturers and distributors' web sites, and peer-reviewed journals as well as other 2007 melamine related pet food contamination messages.

Results: The FDA fulfilled eight of the ten best risk practices. However, 70% (7.9 ± 0.13) of the pet food manufacturers and distributors fulfilled six of the best risk practices while the rest (30%; 2.9 ± 0.18) fulfilled five or fewer (0.4 ± 0.24) of the ten best risk practices. Most (90%) of the institutions collaborated and coordinated with at least a credible source of information and also communicated with compassion, concern, and empathy.

Significance: This study provided an understanding of how adequately the FDA, pet food manufacturers and distributors are able to inform and communicate in a crisis situation that could similarly occur in the human food supply chain.

P3-67 Good Agricultural Practices Online Produce Safety Course

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Introduction: Keeping fruits and vegetables free from contamination by pathogenic organisms is important to the health and safety of consumers. Since the 1970s there has been a steady increase in fresh produce-associated foodborne illnesses. Understanding of food safety issues, food safety practices, and how to reduce microbial risks is extremely important to fresh produce growers, packers and all stakeholders in the produce industry.

Purpose: This presentation will describe the development, implementation, and initial assessment of the Good Agricultural Practices Online Produce Safety Course (GAPsOPSC), an accessible, web-based training program. This course is focused on reducing microbial risks in fresh fruits and vegetables through the education and training of produce industry personnel.

Methods: A Delphi study was conducted with food safety experts in the fields of horticulture, food science, microbiology, education, and veterinary science to determine content priority areas for the course. The GAPsOPSC was developed, utilizing real-world situations related to identified topic areas, so that students can gain knowledge and apply it during course activities and lessons.

Results: Identified topics with a weighted score of 4.0 or above on a 1–5 point scale, with 5 being the most relevant and 1 being the least relevant, were prioritized for inclusion in the course. The results were Irrigation water management (4.8), Development of a food safety plan (4.8), Worker hygiene and training (4.5), Equipment sanitation (4.5), Field sanitation (4.3), Manure and compost handling (4.3), Post-harvest water use (4.2), How to conduct microbiological testing (4.1), Packing facility sanitation (4.0), and Management requirements for food safety (4.0).

Significance: The GAPsOPSC provides consistent, state-of-the-art food safety education and training for produce industry personnel in a format that is interactive and readily accessible, particularly to individuals in rural locations who may otherwise be less likely to receive food safety training.

P3-68 An International Comparison of Food Safety Programs in the Fresh Produce Industry

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Introduction: The globalization of the Canadian supply chain for fresh produce has focused attention on the food safety practices of both Canadian and foreign suppliers at all levels of the chain. HACCP-based food safety programs have been developed by the Canadian Horticultural Council (CHC) for primary producers and packers and by the Canadian Produce Marketing Association (CPMA) for repackers and wholesalers. Other chain participants, including the truckers, food distributors and food retailers, have developed similar programs. These national programs will be recognized by the Canadian Food Inspection Agency.

Purpose: The purpose of this study was to compare the CHC and CPMA programs with food safety programs in countries that export to Canada and to serve as a basis for future buyer specifications for both domestic and imported fresh produce by the members of CPMA, the Canadian Council of Grocery Distributors (national chains) and the Canadian Federation of Independent Grocers (independents).

Methods: The multi-stakeholder steering committee listed priority countries and selected 15 foreign programs for comparison. Two CHC programs (potato and greenhouse) were compared with ten foreign programs (five from the US) and the CPMA program was compared with four (three from the US). Each program's technical content was reviewed in detail against the Canadian base program. A "comparison scale" was established and the final tabulations for industry's use presented the relative "equivalence" of all programs.

Results: Foreign programs that have been developed using the HACCP-based approach adopted

by CHC and CPMA produce comparable food safety practices. Those that do not use this approach, such as California Leafy Greens program, demonstrate significant gaps. Furthermore, the GlobalGAP and GFSI benchmarking schemes generate results similar to the CFIA recognition program. A significant number of exporting countries in the Americas, Africa and Asia are now developing HACCP-based food safety programs for fresh produce.

Significance: This is the first major international comparison of food safety programs in the fresh produce sector. It demonstrates the positive value of adopting the HACCP-based approach. It could serve as a stimulus for the mutual recognition by industry and governments of fresh produce food safety programs for primary producers, packers, repackers and wholesalers or for a new international standard in the fresh produce sector.

P3-69 Evidence for Implicating Food Vehicles in Outbreaks, 1998–2006

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Introduction: Food vehicles implicated in outbreak investigations reported to CDC by state and local health departments are analyzed to help guide foodborne disease control efforts. In 1998, states began reporting in a standard manner the reason a particular food was implicated.

Purpose: To assess the completeness of reporting of reasons for implicating food vehicles.

Methods: We reviewed levels of evidence for implicating a vehicle in foodborne disease outbreaks with > 10 cases reported to the electronic Foodborne Outbreak Reporting System (eFORS) surveillance database from 1998 to 2006. State and local investigators can report one or more of the following levels of evidence for implicating a food item: epidemiologic evidence, laboratory confirmation, compelling supportive evidence, other data, and prior experience.

Results: From 1998 to 2006, 4,851 foodborne outbreaks with more than 10 cases were reported to CDC. In the 2757 (57%) outbreak reports with an implicated food vehicle, 1,308 (47.4%) had epidemiologic evidence without laboratory evidence; 175 (6.3%) had laboratory evidence without epidemiologic evidence; 193 (7%) had both epidemiologic and laboratory evidence; 367 (13.3%) had only other evidence (compelling supportive evidence, other data, and/or prior experience), and 714 (26%) did not provide a reason for implicating a food vehicle. Outbreaks with or without a reason did not differ for the most common etiology, calicivirus, for which 41% provided a reason and 43% did not.

Significance: More than half of the outbreak investigations reported to CDC used either epidemiologic or laboratory evidence to implicate a food vehicle. The fact that over a quarter of the outbreak reports did not supply a reason for implicating a vehicle indicates a need for improvement.

P3-70 Epidemiology of Seafood-Associated Outbreaks in the United States, 1973–2006

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Introduction: Seafood is an important part of a healthy diet; however, risks have been associated with its consumption. Seafood is not uncommonly implicated as a source of foodborne infections and intoxications.

Purpose: The purpose of this study is to describe the epidemiology of seafood-associated foodborne outbreaks.

Methods: We examined reports of foodborne outbreaks investigated by state and local health agencies and reported to CDC during 1973–2006. A seafood-associated outbreak was defined as two or more illnesses due to the consumption of a single seafood commodity (i.e., finfish, crustacean, or mollusk).

Results: Of 24,377 reported outbreaks, 12,257 (51%) implicated a food item; of these, 1,173 (10%) outbreaks were associated with seafood and had a confirmed etiology, causing 9,287 illnesses and 511 hospitalizations. Finfish were the leading source of seafood-associated outbreaks, implicated in 1,034 (88%) outbreaks, 6,210 illnesses and 372 hospitalizations; scombrotoxin poisoning (556 outbreaks [54%], 2,588 illnesses, 116 hospitalizations) and ciguatera (370 outbreaks [36%], 1,437 illnesses, 135 hospitalizations) accounted for most finfish-associated outbreaks. Mollusks, in particular raw oysters, were the second leading source, implicated in 106 (9%) seafood-associated outbreaks; the most common etiologic agents were non-cholera *Vibrio* (36 outbreaks [34%], 1,167 illnesses, 24 hospitalizations) and norovirus (27 outbreaks [25%], 747 illnesses, 16 hospitalizations).

Significance: Understanding the attribution of outbreaks and illnesses to seafood commodities is critical in guiding control efforts. Our data suggest that particular attention should be placed on determining measures to decrease finfish-associated scombrotoxin poisoning and mollusk-associated vibriosis.

P3-71 Food Commodities Associated with *Salmonella* Outbreaks, 1998–2006

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Introduction: The incidence of *Salmonella* infections, which cause an estimated 1.4 million illnesses annually in the United States, has not decreased significantly in the past 10 years. Information on food sources of salmonellosis can inform control strategies.

Purpose: Identify food commodities associated with *Salmonella* serotypes.

Methods: We examined data on confirmed *Salmonella* foodborne outbreaks with two or more illnesses investigated by state and local health agencies and reported to CDC during 1998–2006. Food items implicated in outbreak investigations were classified into one of twelve major food commodity categories that correspond to their essential component.

Results: During 1998–2006, 999 *Salmonella* outbreaks causing 26,939 illnesses, 2,565 hospitalizations, and 38 deaths were reported to CDC. For 634 (63%) outbreaks, an implicated food item was reported; 351 (35%) outbreaks and 11,086 illnesses were associated with foods that could be assigned to a single commodity. Food vehicles in these outbreaks represented 11 of the 12 commodity categories; the top three commodities, eggs (27% of outbreaks, 23% of illnesses), poultry (26% of outbreaks, 18% of illnesses), and produce, which combined the fruits-nuts and vegetables commodities (19% of outbreaks, 33% of illnesses), comprised the vast majority. Outbreaks associated with eggs were due predominantly to *Salmonella* serotype Enteriditis (76%), whereas outbreaks associated with poultry were caused by 26 *Salmonella* serotypes, most commonly Enteritidis (20%), *S.* Typhimurium (15%) and Heidelberg (12%) and outbreaks associated with produce were caused by 21 serotypes, most commonly,

Newport (18%), Enteritidis (16%), Typhimurium (9%), and Javiana (7.5%).

Significance: Over 70% of *Salmonella* outbreaks were due to eggs, poultry, and produce, and one-third were due to serotypes Enteritidis and Typhimurium. Control efforts targeting these foods and serotypes would impact more than half of foodborne *Salmonella* outbreak-related illnesses.

P3-72 Enteric Disease Outbreaks Associated with Fairs and Festivals, 1998–2006

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Introduction: Enteric disease outbreaks associated with fairs and festivals in the United States present special challenges. The epidemiology of such outbreaks has not been described. The Centers for Disease Control and Prevention (CDC) conducts surveillance for enteric disease outbreaks investigated by local and state health departments in the United States.

Purpose: We describe the epidemiology of outbreaks associated with fairs and festivals to understand how to prevent illness at such common events.

Methods: We reviewed data from CDC's Foodborne and Waterborne Outbreak Surveillance Systems for 1998–2006 and conducted a literature search. A fair/festival associated outbreak was defined as: two or more illnesses related to attendance at temporary public gatherings, with outside food vendors, for the purpose of exhibiting animals or agricultural goods, or for holding artistic performances, dances, or exhibitions.

Results: Among 11,630 foodborne, waterborne, and animal contact associated outbreaks reported during 1998–2006, 95 (0.82%) outbreaks, 4,467 (1.8%) illnesses, and 3 (1.3%) deaths were associated with fairs/festivals; the median size (17 illnesses) was greater than the median size of non-fair/festival outbreaks (7 illnesses). Of the 95 reported fair/festival outbreaks, 79 (83.2%) were foodborne, 12 (12.6%) were associated with contact with animals, 3 (3.2%) were waterborne, and 1 (1.1%) was due to person-to-person transmission. The most commonly reported etiologies were enterohemorrhagic *Escherichia coli*, which was responsible for 19 (20.0%) outbreaks, 12 (63.2%) of which were associated with animal contact, specifically animal exhibits and petting zoos; and Norovirus and *Salmonella*, which caused 19 (20.0%) and 13 (13.7%) foodborne outbreaks, respectively.

Significance: Fairs/festivals are important sources of enteric disease outbreaks and these outbreaks are generally larger than those in other settings. The modes of transmission in these outbreaks indicate that efforts of health agencies, animal exhibitors, and venue operators should include: exclusion of ill foodworkers, safe food and water supplies, training of food handlers, and adequate hand-washing facilities.

P3-73 Contributing Factors Identified in Outbreaks from CDC's National Electronic Foodborne Outbreak Reporting System, FoodNet Sites, 2006

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Introduction: Outbreak investigations are important for understanding the epidemiology of foodborne pathogens.

Purpose: Information on contributing factors can lead to the identification of new hazards or unsafe handling practices, findings which, in turn, may lead to new prevention measures.

Methods: We analyzed data from CDC's national electronic Foodborne Outbreak Reporting System (eFORS) and supplemental forms completed by states participating in FoodNet for 2006. Contributing factors (CFs) were defined as food safety practices and employee behaviors most associated with foodborne illness outbreaks.

Results: FoodNet sites reported 301 foodborne outbreaks in 2006. Of these, 195 (65%) were restaurant-associated and 114 (58%) had ≥ 1 CF identified. A supplemental CF form was submitted to FoodNet for 102 outbreaks, including Norovirus (37%), *Salmonella* (22%) and *C. perfringens* (12%). The three most commonly reported CFs were: handling by an infected person or carrier of a pathogen (76%), bare-handed contact by food worker (38%), and raw product contaminated by pathogens from animal or environment (22%). CFs were identified by both epidemiologists and environmental health specialists in 48 (47%) outbreaks and by epidemiologists alone in 42 (41%) outbreaks. Most CFs were identified following an interview with an operator or foodworker (67/102 outbreaks (66%); an environmental or food sample culture was used to determine a CF in 18 (18%) outbreaks; 17/102 (17%) of contributing factors were reported based on symptom profile only or were assumed, based solely on etiology.

Significance: Proper hand hygiene during food preparation should be a continued focus of public health education messages. It is critical that epidemiologists and food regulatory personnel work together to identify factors contributing to restaurant-associated outbreaks. Ensuring consistent and reliable reporting of contributing factors is important in developing appropriate intervention and prevention strategies.

P3-74 Invasive *Salmonella* Infections in the United States, 1996–2006

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Introduction: It is estimated that *Salmonella* infects ~1.4 million persons each year in the United States, requiring 15,000 hospitalizations and causing 400 deaths. *Salmonella* infections most often cause mild-to-moderate gastroenteritis that resolves without treatment; however, invasive *Salmonella* infections can be severe and potentially life threatening.

Purpose: The objective of this study is to describe the epidemiology of invasive *Salmonella* infections in the United States from 1996 to 2006.

Methods: We analyzed data on human *Salmonella* isolates serotyped in state public health laboratories and reported to CDC during 1996–2006. A case of invasive infection was defined by isolation of *Salmonella* from blood, cerebral spinal fluid, or other normally sterile site, excluding urine.

Results: During 1996–2006, 23,101 (5.9%) of 390,741 *Salmonella* infections were invasive; of these, 22,020 (95.3%) isolates were from blood, followed by 350 (1.6%) gall bladder, 218 (0.9%) joint, 157 (0.7%) cerebral spinal fluid, and 350 (1.6%) from other sites. Median age of patients with invasive isolates was 34 years (range 0-103), with 22% <1 and 21% 60 years or older; less than half (45.3%) were female. 43% of infections occurred between June and September. Among invasive infections, the five most frequently reported serotypes were Enteritidis (18.2%), Typhimurium (14.7%), Typhi (11.3%), Heidelberg (10.0%), and

Poster

Paratyphi A (3.6%), while Typhi and Paratyphi A are more common causes of invasive disease.

Significance: Invasive *Salmonella* infections, primarily bacteremia, remain a public health problem, especially in infants and elderly persons. Prevention efforts should target these populations, and more research is needed to understand risk factors and serotype-specific pathogen characteristics leading to invasive disease.

P3-75 Estimation of the Burden of Diarrheal Diseases in Miyagi Prefecture, Japan, 2005–2006

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Introduction: In Japan, under the Food Sanitation Law, the numbers of food poisoning cases must be reported; however, this does not exactly reflect the real burden of foodborne illness because of the passive nature of this surveillance. We have been estimating the real burden of diarrheal diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in one Prefecture where data on laboratory-confirmed infections is available.

Purpose: The purpose of this study was to estimate the burden of illness associated with *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Miyagi Prefecture, in the northern part of Japan (population: 2.36 million).

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories in Miyagi Prefecture from April 2005 to March 2007. The stool sampling rate and physician consultation rate were estimated from a population telephone survey conducted in the same prefecture, for a two-week period each in different seasons. The two rates were multiplied to the laboratory-confirmed cases. Each factor was introduced in our Monte-Carlo simulation model as a probability distribution and ran for 10,000 iterations.

Results: The physician consultation rate and the stool sampling rate were estimated from the combined telephone survey data as 32% and 11%, respectively. Consequently, the estimated mean numbers of illnesses in this region for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* were 35,684; 4,939; and 2,368 in 2005 and 37,901; 2,829; and 1,779 in 2006; respectively. The number of cases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* reported to Miyagi Prefecture were 143, 12 and 32 respectively in 2005; 109, 11 and 0 respectively in 2006.

Significance: These data reveal that significant difference between our estimate of burden of illness and the reported foodborne disease cases associated with the three pathogens. Furthermore, estimations from multiple years will enable us to discuss the trends for each pathogen in the future.

P3-76 Assessing Food Safety Trends in Mexican Food

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Introduction: Increases in the ethnic population of the United States have increased Americans' exposure to foods from other cultures, making ethnic foods one of the fastest-growing areas of the food industry. An increase in foodborne illnesses related to ethnic foods was observed during 1990–2000. Mexican food is one of the most frequently reported sources of outbreaks.

Purpose: This study further extends the compilation of the CDC data from 2001–2005 and examines and characterizes food safety risks and trends related to Mexican food over an extended period. The data will be useful for determining specific risks related to Mexican foods.

Methods: Foodborne illness data (2001–2005) was obtained from the CDC database (http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm), and ethnic foods were grouped as previously described by Simonne et al. (2004). Data were cross checked with other publications whenever possible. Combined data for Mexican foods (1990–2005) were processed and ranked based on number of outbreaks, etiology, outbreak location and outbreak vehicle.

Results: Outbreaks implicating Mexican foods increased from 2% of cases in 1990 to 5% of cases in 2005. Overall, 526 outbreaks (17,824 cases) were reported during the period. 36% had a known etiology, while 64% did not. Outbreaks occurred at restaurants (47%), private homes (13%), schools (6%), and other locations (4%). *Salmonella* spp. (38%), *Clostridium perfringens* (22%), *Norovirus* (12%), *Shigella* (7%) and *Staphylococcus* (6%) were the major known etiologies. The top five vehicles for outbreaks with known etiologies were tacos (16%), chili (10%), salsa (10%), refried beans (8%), burritos (5%), and guacamole (4%). Trends in etiologies, locations, and vehicles were similar to those reported in 2004 by Simonne et al., except for findings on *Norovirus*.

Significance: This study reveals that cases of foodborne illness involving Mexican foods seem to have observable trends for etiology and vehicle. The study will provide valuable risk assessment tools.

P3-77 Microbiological Quality of Eggs in Six States of Mexico

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Introduction: International episodes of human salmonellosis associated with the consumption of eggs or products thereof contaminated with *Salmonella* Enteritidis have resulted in a renewed interest in determining the microbiological quality of eggs. *Salmonella* can contaminate the contents of clean, intact shell eggs as a result of infections of the reproductive tissue of laying hens, or at any point in the food chain.

Purpose: Since little information is available on the current prevalence of bacterial flora on retail eggs in Mexico, this survey was prompted to analyze the microbiological quality of retail eggs in six different regions of Mexico.

Methods: A total of 180 eggs purchased from local supermarkets in six States in Mexico (Coahuila, Chihuahua, Hidalgo, Nuevo León, Querétaro and Puebla) were analyzed for microbiological quality. Shells (180) and liquid whole eggs (180 samples) were examined for coliforms, *Escherichia coli* and *Salmonella* spp., using the Mexican Official Methods and the Bacteriological Analytical Manual (BAM) protocols.

Results: In liquid whole egg (yolk and albumen), coliforms were found in 9 (5%) samples at wide range of counts, from 4 to 500 MPN/g. Only one (0.5%) sample was positive for *E. coli* and *Salmonella* spp. When shell eggs were analyzed, results showed that 19 (10.5%) shells had coliforms, mainly at low levels (1.2 to 9 MPN/shell). *E. coli* and *Salmonella* spp. were detected only in two (1.1%) and one (0.5%) samples, respectively.

Significance: Previous reports of other countries have shown that the incidence of *S.* Enteritidis in egg layer flocks usually ranges from 0.1 to 1.0%. In this study, similar levels of *Salmonella* were detected in samples of retail eggs distributed in Mexico, and counts of coliforms and *E. coli* were also very low.

P3-78 Establishing a Rep-PCR DNA Fingerprinting Library for *Escherichia coli*

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Introduction: Enterovirulent *Escherichia coli* (EEC) is one of the most common causative agents of gastroenteritis and one of the most frequent community acquired pathogens in humans. Pathogenic *E. coli* strains belong to a few subgroups among the total *E. coli* population, which is comprised of five major phylogenetic lineages, as revealed by the well-characterized ECOR collection of *E. coli* isolates. Laboratory detection of pathogenic strains is typically done by time-consuming biochemical assays or DNA probes, which often require prior knowledge to determine virulence and serotype. Often, a combination of assays is required for proper identification.

Purpose: This study reports the creation of an *E. coli* library to be applied with the DiversiLab System™ for use in strain typing for epidemiology and surveillance purposes.

Methods: Over 100 previously characterized *E. coli* isolates (including the complete ECOR collection, and a number of other EEC isolates of varying pathotype) were cultured. Purified DNA from these isolates was amplified, using the system *Escherichia* Kit for DNA fingerprinting. The amplified product was separated with use of microfluidics-based detection. The resulting data was analyzed by using the system software, and an *E. coli* library was generated.

Results: In general, the library shows clustering on the basis of serotype and pathotype. Serotypes that occur among the different pathotypes show different rep-PCR fingerprint patterns. The library discriminates each pathotype such that it clusters exclusively with samples of the same pathotype. The pathotypes exhibit multiple fingerprints, indicating strain discrimination within these groups. The members of the ECOR collection also cluster according to their respective phylogenic lineages. With few exceptions, most serotypes cluster together.

Significance: The data indicate that *E. coli* strain typing with a characterized library may provide useful additional information or confirmation of sero- and/or pathotype. The DiversiLab System, with the inclusion of reference libraries and database-building capabilities, is a useful tool to aid surveillance when conducting epidemiological studies.

P3-79 Multiplex Ready-to-Use PCR Assays for the Detection of STEC and the Identification of EHEC

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Introduction: Shigatoxins-producing *Escherichia coli* (STEC) have clearly emerged as important life-threatening foodborne pathogens because of their implication in several human outbreaks in various countries worldwide. Among the STEC, the Entero-hemorrhagic *E. coli* (EHEC) are associated with Hemolytic Uremic Syndrome (HUS), a disease resulting in serious kidney disorders which may lead to death. The most prevalent *E. coll* serovars associated with HUS are O26, O103, O111, O145 and O157H7. These strains carry virulence-associated genes, encoding for the shigatoxins (stx_1 and/or stx_2), the intimin (*eae*) and/or the enterohemolysin (ehxA).

Purpose: Real time PCR assays using these targets have been shown to be relevant for the detection of STEC in food samples, and specific PCR tests have been developed for the identification of the 5 major O-serogroups (Perelle et al., 2007). However, detection and identification of such a variety of strains require the use of many primer/probes. Two innovative multiplex PCR assays, using a new real time PCR technology (the GeneDisc technology), have been developed for the routine detection of STEC and the identification of EHEC in foodstuff.

Methods: The first assay detects the presence of STEC and *E. coli* O157 strains, by combining multiplex PCR for *stx*, *eae*, *ehx* and *rfb*E. The second assay allows the identification of the 5 major EHEC serogroups by targeting genes wzx (O26 and O103), wbdI (O111), ihp1-like (O145) and fliC (*E. coli* H7).

Results: The first assay validation (STEC and O157), run with more than 200 *E. coli* strains, showed more than 99% compliance. All *stx* and *eae* variants were analyzed, and only strains carrying *stx*2f (3/3) and *eae*-rho (1/4) variants were not detected. For the second assay (EHEC), all O157:H7 and their O-rough derivatives were detected by combining the O157*rfb*E and fliC-H7 PCR. The O26, O103 and O111 PCR were shown to be 100% sensitive and specific. O-rough mutants for each serogroup had been correctly identified. For O145 PCR, serogroups O133 and O137 showed cross reactions, but both were *stx* and *eae* negative.

Significance: In conclusion, both GeneDisc assays offer reliable tools for the routine screening of STEC and EHEC in bacterial isolates and in samples containing mixed cultures of bacteria.

P4-01 Withdrawn

P4-02 Withdrawn

P4-03 DSC Development of Aerobic Enrichment Broth for Isolation of *Campylobacter jejuni* and *Campylobacter coli* from Foods

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Introduction: Foods of animal origin have been identified as significant sources of *Campylobacter*. Unfortunately, the microaerophilic nature of campylobacters has complicated its isolation from diverse matrices, including foods and feces.

Purpose: To develop an alternative enrichment broth for *Campylobacter jejuni/coli* that does not require microaerobic incubation

Methods: At least six different formulations were originally designed, based on the Blood-Free Enrichment Broth (BFEB) proposed by Tran (1998). Of these, modified BFED (M-BFEB) was the most effective in recovering campylobacters. The M-BFEB does not contain charcoal. Detection limits for M-BFEB were determined and compared with conventional enrichment broths (Bolton, BFEB and Preston), using several *C. jejuni/coli* strains either alone or as mixed cultures with *E. coli.* Pure cultures (10–10^8 CFU/ml) were inoculated into M-BFEB, BFEB, Preston, and Bolton. Also, these strains were inoculated into retail pork meat (10–10^8 CFU/g), enriched, and incubated (42°C, 24 and 48 h) in either 5% or 21% oxygen. Rates of recovery after enrichment for different broths in the two atmospheres were determined.

Results: Of all new formulations tested, charcoal-free M-BFEB in atmospheric oxygen allowed the best recovery of *C. jejuni/coli.* In co-culture, *C. jejuni/coli* were recovered even though *E. coli* was present in high levels (10^6 CFU/ml). The minimum detection limit of M-BFEB for pure cultures of *C. jejuni/coli* was 10^0 CFU/ml, which was similar in artificially inoculated pork meat. The results were similar to those seen with original BFEB media (< 10^0 CFU/ml) and better than those seen with Preston broth (> 100 CFU/ml). Although Bolton broth was the best medium (< 10 CFU/ml), it requires microaerobic incubation.

Significance: Overall, the efficiency of recovery *C. jejuni/coli* in M-BFEB in an aerobic atmosphere compared favorably with that obtained under microaerobic incubation with other media. Also, M-BFEB did not require the use of blood or charcoal to reduce oxygen tension, which could favor immediate analysis by PCR.

P4-04 Novel Method for Screening Raw Seafood for *Vibrio parahaemolyticus*

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Introduction: The conventional detection method for *Vibrio parahaemolyticus* is laborious and requires 3 days to obtain results. The Compact Dry VP method has been developed to screen raw seafood for *V. parahaemolyticus* simply and rapidly.

Purpose: The aim of this study was to evaluate the novel method for screening raw seafood for *V. parahaemolyticus.*

Methods: Compact Dry VP contains a dried medium with a specific chromogenic substrate. *V. parahaemolyticus* will appear as blue colonies after 18–20 h incubation at 35°C. The novel method was compared with FDA-BAM method. Five samples of two fresh and two frozen raw seafood types were artificially contaminated with *V. parahaemolyticus.* The artificially contaminated food lots were prepared at three (low, medium, and high) contamination levels. A one-way analysis of variance (ANOVA) was performed to determine the differences in results between the two methods. The slope, intercept, and square of the linear correlation coefficient (r^2) were calculated to plot the results from Compact Dry VP and FDA-BAM methods.

Results: The r^2 between the novel and FDA-BAM methods were 0.99 for fresh raw tuna, 0.95 for fresh raw oysters, 0.95 for frozen raw salmon, and 0.95 for frozen raw scallops. For all comparisons, the slope and intercept values, determined by linear regression analysis, were close to 1.00 and 0.00, respectively. There was not a significant difference between the two methods by one-way ANOVA.

Significance: It was concluded that the novel method was useful for the rapid screening of fresh and frozen raw seafood for the presence of *V. parahaemolyticus.*

P4-05 Evaluation of a Rapid Agarose Gel Electrophoresis System for Detection of *Shigella* Species in Foods

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Introduction: In the interest of public safety, regulatory laboratories responsible for foodborne pathogen detection strive to minimize time required for testing. Rapid agarose gel systems have recently been marketed that reduce electrophoresis time from hours to minutes. If such rapid systems do not compromise the quality of test results they could benefit regulatory laboratories by decreasing test turn-around time.

Purpose: The purpose of this study was to evaluate a rapid electrophoresis system and determine whether it may be an acceptable alternative to conventional electrophoresis systems for detection of *Shigella* species by PCR.

Methods: The FlashGel® System by Lonza Rockland, Inc. was compared to a conventional agarose gel system for analysis of PCR products resulting from screening broth cultures for the presence of *Shigella* species. Qualitative comparisons and two quantitative comparisons were conducted: 1) serial dilution of PCR amplicon prior to electrophoresis, and 2) serial dilution of *Shigella* broth cultures prior to PCR testing.

Results: The rapid gel system was found to be very fast and easy to use, and it enabled visualization of DNA migration in real time, although amplicons were not as distinct in appearance as with the conventional gel system. Detection limits of 0.24 ± 0.12 ng and 0.48 ± 0.23 ng of *Shigella* PCR amplicon were observed for the rapid system (5 µl/lane) and the conventional system (10 µl/lane), respectively (n = 4). Both systems enabled detection of *Shigella* amplicon if > or = 10 CFU/m l (n = 4) of *Shigella* species were present in the test culture.

Significance: These data suggest that the FlashGel® system for rapid electrophoresis is a suitable time-saving alternative to conventional gel electrophoresis for detection of a 629 bp *Shigella* PCR amplicon.

P4-06 A PCR-Based Method to Distinguish Viable from Non-Viable Spores of *Bacillus subtilis*

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Introduction: The long-term persistence of DNA molecules has created concerns about the relationship between the detection of DNA by PCR and its relationship to bacterial cell viability. Intercalating dyes such as propidium monoazide (PMA) have been shown to selectively penetrate the membranes of dead cells, bind to DNA, and prevent its amplification. PMA-based methods have been applied to distinguish live from dead cells of various Gram-negative bacteria, but have yet to be applied to sporeformers.

Purpose: The purpose was to develop a technique using PMA coupled with quantitative real-time PCR

(QPCR) that is able to distinguish between viable and non-viable spores of *Bacillus subtilis*.

Methods: Various concentrations of PMA (1– 25 µM) were added to live spores and those inactivated by autoclaving, followed by a variety of incubation periods (0–60 min at room temperature). After photolysis, DNA was extracted from the spores by bead beating and isopropanol precipitation. A TaqMan QPCR assay targeting the spoOA gene was designed for enumeration of *B. subtilis* DNA copy number.

Results: The QPCR assay was able to detect as few as 10 spores per reaction, with log-linear detection in the range of 10^1–10^6. When the optimized PMA treatment (10 µM PMA for 50 min) was applied to live and dead spores prior to DNA extraction, the resulting QPCR amplification efficiency of DNA extracted from live spores remained unchanged. On the other hand, the PMA treatment prevented the amplification of DNA derived from autoclaved *B. subtilis* spores. In mixtures containing differing proportions of viable and thermally inactivated spores, it was possible to detect as few as 10^2–10^3 viable spores in a background of 10^7 inactivated sp.

Significance: DNA intercalating agents can be used to distinguish between live and inactivated spores using QPCR, providing a promising molecular approach to assess spore viability.

P4-07 Validation of a PCR-Based Protocol for the Rapid Detection of *Staphylococcus aureus* from Infant Formula, Soy Protein Isolate and Ground Beef

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Introduction: Food, drug, and cosmetic manufacturers test for *Staphylococcus aureus* as a quality indicator and because of the pathogenic nature of this species.

Purpose: This study tested the efficacy of screening laboratory-contaminated infant formula and soy protein isolate, and naturally contaminated ground beef, using the BAX® system Real-Time PCR assay for *S. aureus*.

Methods: Soy- and milk-based infant formulas and soy protein isolate destined for incorporation into infant formula were spiked with low levels of *S. aureus* and allowed to equilibrate. A *S. aureus* positive lot of ground beef was purchased at retail and diluted with uncontaminated ground beef to give fractional positive results. The BAX® System method was compared with the ISO 6888-3 and 6888-1 methods for detecting *S. aureus* in infant formula and ground beef. Soy protein isolate testing was compared with the FDA-BAM method.

Results: Soy-based infant formula studies demonstrated 12/20 spiked samples positive by both culture and PCR methods. Milk-based infant formula demonstrated 10/20 and 17/20 positive by culture and 10/20 and 19/20 positive by PCR in two trials. For the ground beef study, both culture and PCR methods demonstrated 18/20 enriched samples positive, and for the soy protein study, both methods showed 16/20 samples to be *S. aureus* positive. All PCR positive enrichments were culture positive. Additionally, an alternative enrichment for infant formula significantly improved the sensitivity of the assay.

Significance: This study indicated that PCR detection of *S. aureus* is rapid and sensitive. Test kit results demonstrated either no significant difference or a statistically greater sensitivity than the reference culture methods. As a genetic method, this assay reduces decision times by eliminating biochemical confirmation steps required in the ISO and FDA-BAM methods

P4-08 An Independent Laboratory Evaluation of a Real-Time PCR Method Utilizing a Single Nonspecific Enrichment to the USDA/FSIS and FDA/BAM Reference Methods for the Detection of *Escherichia coli* O157:H7 in Selected Foods

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Introduction: Despite regulatory efforts to improve food safety, *E. coli* O157:H7 continues to be an important issue for the US food supply. In 2007, a total of over 29 million pounds of ground beef was recalled because of this pathogen. The use of molecular methods, such as real-time PCR, has increased in recent years for food safety. Specific oligonucleotide probes known as molecular beacons increase specificity of an assay.

Purpose: The purpose of this study was to compare the performance of iQ-Check *E. coli* O157:H7 to the USDA/FSIS reference method for the detection of *E. coli* O157:H7 in ground beef and the FDA/BAM reference method for the detection of *E. coli* O157:H7 in apple cider and fresh spinach.

Methods: Three protocols were tested in this study: an 8 h enrichment in Buffered Peptone Water (BPW), 24 h enrichment in BPW and testing the reference method enrichment with the PCR test kit. Chi-square analysis was employed to determine if a statistical difference existed between the methods. All samples were tested with another commercially available PCR test and with a lateral flow device.

Results: Overall method agreement was 90%. The test method identified more true confirmed positive samples than the reference method. Unlike the reference method, with which some difficulty occurred during attempts to select isolated colonies, specifically from raw ground beef, the test method performance is independent of the concentration of interfering flora. The performance of the test method using BPW was superior to that of the two commercial methods using reference method enrichments.

Significance: Early and accurate detection of pathogenic *E. coli* O157:H7 is important from a public health and food safety perspective. The use of real-time PCR utilizing a single nonspecific 8 h enrichment in BPW is a useful tool to help ensure safe food.

P4-09 Automatic Multiplex Real-Time PCR System for the Fast Detection of Twelve Foodborne Pathogens on One 96 Well Plate

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Introduction: Development of more rapid and easier methods of simultaneously detecting many more bacteria without false positive results is significant because such methods offer the opportunity to increase the efficiency of food pathogen testing.

Purpose: An automatic multiplex real-time PCR system has been developed for the specific detection of 12 representative pathogens — *C. jejuni*, *E. coli*

O157:H7, *S. aureus*, *B. cereus*, *V. parahaemolyticus*, *L. monocytogenes*, *Y. enterocolitica*, *Salmonella* spp., *Shigella* spp., *V. vulnificus*, *C. perfringens*, and *V. cholera* — responsible for foodborne disease in a variety of foods, including raw, Ready-to-Eat, and frozen foods.

Methods: The assay targets each bacterial specific toxin gene or others (hipO, *stx*1, *stx*2, femA, groEL, toxR, iap, 16s rDNA, invA, iapH, vvh, alpha-toxin, and ctx). To allow multiplex detection of the 12 pathogens, specific primers and TaqMan probes labeled with the various fluorescent markers were designed. An internal positive control was developed for the 12 pathogens plus/minus assay and single and multiplex assay were performed and optimized on AB7500. Furthermore, Automatic real-time PCR Analysis Software was developed that can analyze data extracted after the real-time PCR run.

Results: Ultimately, the assay was constructed so that twelve pathogens can be simultaneously detected on one 96 well plate. Exclusivity and inclusivity were confirmed by using template DNA from fifty bacterial strains and the relative limit of detection for the single assays was found to be 10 pg of purified bacterial genomic DNA. Automatic analysis software is an integral part of the developed detection system and can decide which pathogen is present as a contaminant within 5 min. Overall, the developed system is reliable and easy to use, and the entire procedure can be completed in less than 2 h.

Significance: The automatic multiplex real-time PCR system can simplify food testing and be efficiently applied in case of multiple infection. These findings will assist food safety and the regulatory system in establishing an early screening method, thereby decreasing the incidence of food poisoning outbreaks.

P4-10 Evaluation of a Molecular Beacon Real-Time PCR Assay for Detection of *Listeria monocytogenes* in Selected Foods from a Single Primary Enrichment

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Introduction: Listeria monocytogenes is an important concern in food safety because of its high mortality rate (30%), with 90% of all reported cases resulting in hospitalization. Conventional methods for detection of *L. monocytogenes* in contaminated food take a minimum of 3–7 days to obtain results. In recent years, the use of PCR technology has offered users a rapid method for detection of a target with the increased specificity that comes with using probes specific for the DNA of certain target bacteria.

Purpose: iQ-Check *Listeria monocytogenes* II is a Real-Time PCR kit utilizing specific oligonucleotide probes, called molecular beacons, to detect *L. monocytogenes* in food. The test method allows for the detection of a presumptive result from a food sample in 24 h, utilizing a single primary enrichment in *Listeria* Special Broth. This work is a validation of the test method for detection of *L. monocytogenes* from deli turkey, hot dogs, cottage cheese and smoked salmon.

Methods: An inclusivity and an exclusivity study were performed to test the sensitivity and specificity of the test. Method comparison studies involved inoculating food with a low level of cells (< 5 CFU/25 g) and comparing results to the specific reference method for that food, specifically the USDA/FSIS Microbiology Laboratory Guidebook, FDA Bacteriological Analytical Manual or AOAC Official Method. A Chi-square analysis according to Mantel-Haenszel was employed to determine if a statistically significant difference resulted from the comparison of the test and reference methods.

Results: Inclusivity and exclusivity rates were 100%. Overall method agreement between the test and reference method was 95%, with the test method identifying more true confirmed positive samples than the reference method. No significant difference was observed between the methods.

Significance: Real-time PCR technology is a useful tool to help ensure food safety. The new assay shortens the length of time necessary for detection of the harmful pathogen *L. monocytogenes*.

P4-11 DSC Enzymatic Release of DNA from Fusarium Spores for Use in Real-Time PCR

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Introduction: Fusarium verticillioides and *Fusarium graminearum* are found in agricultural commodities such as corn; therefore, spores of Fusaria can be present in food in low numbers. *Fusarium* spores in food could germinate during storage or processing to produce heat stable mycotoxins, which cause adverse health effects in humans and animals. Rapid detection of *Fusarium* spores by molecular methods requires spore lysis, with minimal destruction of the spore DNA.

Purpose: The purpose of this study was to examine the effectiveness of three enzymes for extracting DNA from low levels of spores of *Fusarium verticillioides* and *Fusarium graminearum* that were used in real-time PCR detection.

Methods: Fusarium spores were mixed with enzymes dissolved in their respective buffers and incubated at 37°C for 4 and 6 h for *Fusarium graminearum* and *Fusarium verticillioides*, respectively. Following incubation, spore digestion mixes were shaken in the FastPrep® homogenizer. DNA was extracted from the supernatant by use of a DNA binding resin and mini-columns, and was analyzed in real-time PCR, using species-specific primers and probes developed in the lab.

Results: Lysis with β(1→3)-glucanse from *Arthrobacter luteus* (lyticase) was the most effective enzyme because 10 spores of *Fusarium graminearum* (average cycle threshold (Ct 35.7 ± 1.86, n = 4) and *Fusarium verticillioides* (Ct 36.05 ± 0.21, n = 4) were detected. After lysis with chitinase from *Streptomyces griseus*, as few as 100 spores of *Fusarium verticillioides* (Ct 37.73 ± 0.98, n = 6; three not detectable) and *Fusarium graminearum* (Ct 37.84 ± 1.14, n = 4; one not detectable) were detected. Lysates after digestion with enzymes from *Trichoderma harzianum* or Glucanex® had up to 1000 spores for *Fusarium graminearum* (Ct 36.64 ± 1.65, n = 4) and *Fusarium verticillioides* (Ct 37.23 ± 1.09, n = 4).

Significance: Based on these results from enzymatic digestion, a sensitive method was developed for detecting low numbers of Fusarium spores from various matrixes, including food.

P4-12 Development of Conventional PCR Method to Detect the Presence of Ara h 1 Peanut (*Arachis hypogaea*) Allergen in Food

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Introduction: People suffering from food allergies are dependent on accurate food labelling. Hidden pea-

nut allergens in food products are a serious problem, mainly for peanut allergic consumers. Undeclared peanut traces can be found in processed food products very often so it is necessary to develop sensitive and reliable methods to detect such contaminants.

Purpose: The purpose of this study was to develop a conventional PCR method for the detection of peanut allergens in food products.

Methods: The conventional PCR method for the detection of peanut specific DNA (Part of the coding region Ara h 1) was developed with the detection limit of less than 0.1%. The primer pairs were designed to amplify part of the sequence of the Ara h 1 gene, and the size of the amplification product was 216 bp. The designed primers yielded just peanut Ara h 1 DNA specific amplification product.

Results: Twenty samples from the market with peanuts declared were tested by this method. In all cases, peanut allergens were detected in conformity with the declared composition. The specificity testing did not show any cross reactivity with 21 common food ingredients.

Significance: This conventional PCR method was able to detect peanut allergens in peanut-containing products in the market of the Czech Republic. Monitoring of various food commodities without peanut package declaration will be desirable.

P4-13 Internal and Independent Laboratory Validation of a Reverse-Transcriptase PCR Assay for Detection of Genus *Listeria* from Stainless Steel Surfaces

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Introduction: Listeria spp. detection and monitoring is important in food processing plant safety management because it can be an indicator of possible *L. monocytogenes* contamination. Sensitive methods that facilitate faster time to result are needed so that intervention strategies may be implemented more rapidly.

Purpose: We hypothesized that we could develop a more sensitive/rapid assay for genus *Listeria* detection, using Reverse Transcriptase PCR (RT-PCR). The goal was to develop an assay using RT-PCR that would detect genus *Listeria* from environmental samples in < 8 h.

Methods: Spiked surface studies were conducted to compare an RT-PCR assay for genus *Listeria* against the USDA-FSIS reference culture method. *L. ivanovii, L. monocytogenes*, and *L. innocua* were used as test organisms for the internal studies, and *L. ivanovii* was used for the external lab studies. Competitive flora were also applied for some studies. Twenty spiked replicates and five unspiked controls were tested by both the test and reference method. In addition, 58 *Listeria* and 52 non-*Listeria* were tested for inclusivity and exclusivity.

Results: The results of the internal studies showed that the RT-PCR method, across all three surfaces and organisms, detected 100/100 samples in < 8 h compared to 51/100 for the reference method, which required ~5 days for final results. Ninety-eight of the 100 samples positive by RT-PCR were culture-confirmed as *Listeria*. The results of the external studies showed that the RT-PCR method detected 20/20 versus 5/20 for the reference method. Of the 20 RT-PCR positives, 12 were culture-confirmed as *Listeria*. All unspiked samples, for both the internal and external evaluations, were negative by RT-PCR and culture. The RT-PCR method detected 58/58 *Listeria* strains and did not detect any of 52 non-*Listeria* sp.

Significance: The aggregate data indicate that the RT-PCR method was statistically superior ($\chi^2 = 56.7$) to the reference method. In addition to superior performance, results were obtained in < 8 h.

P4-14 Methods for the Characterization of Bacterial Starters Used in Food Applications

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Introduction: We have developed a range of molecular and physiological tools to study and enhance the development and the adaptation of bacteria in different industrial environments, by improving their viability and activity. These technical tools allow a better understanding and control of the probiotics or other types of bacterial starters inoculated in hostile environments.

Purpose: The purpose of this study was to develop a method allowing the measurement of the level of adaptive state for bacterial starter production.

Methods: Different strains of *Lactobacillus* spp. and *Bifidobacterium longum* were grown in MRS broth pH 6.5 (optimal conditions) or in acidic conditions in MRS broth at pH 4.5 to 3.5 (adapted cells). After incubation at 37°C for 16 h, the cultures were further characterized: (i) for survival at low pH into potassium phosphate buffer 100 mM, (ii) for regulation of intracellular pH homeostasis and (iii) for expression of several stress genes.

Results: These methods allowed us to predict bacteria survival and activity under harsh environment conditions, particularly under acidic conditions. Various pre-adaptation conditions were tested to select the best one, which is necessary to stimulate expression of specific stress genes and to induce physiological changes in the cell. We showed a correlation between the value of the intracellular pH of cultures and the ability to tolerate low pH. The same correlation was demonstrated between the expression of stress genes and the low pH tolerance.

Significance: The approach, which focused on the optimization of the performances of bacterial starter in food manufacturing, could be applied for studying antagonistic bacterial features in order to develop new approaches for the prevention, sometimes eradication, of pathogenic bacteria in various industrial environments.

P4-15 DSC Identification of Primers to Detect *Lactobacillus acidophilus* NP51 in Cattle Feces

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Introduction: Supplementation of cattle diets with *Lactobacillus acidophilus* strain NP51 decreases shedding of *E. coli* O157 up to 50% during feeding. The decrease of *E. coli* O157 in feces and hides of finishing cattle could lead to a decrease in contamination and a safer product. Therefore, the need exists to verify that NP51 has been fed to cattle. However, with multiple organisms in manure, a genetic-based identification system is necessary.

Purpose: The purpose of this study was to identify primers to develop a quantitative PCR method to detect and enumerate NP51 in cattle feces.

Methods: DNA from NP51 culture and 38 isolates from cattle fecal samples isolated on MRS agar was subjected to PCR amplification of 16S-23S

intergenic spacer region using universal primers, 5'-GAATCGCTAGTAATCG-3' and 5'-GGTTCCCCCAT-TCGGA-3' that anneal within the 16S and 23S region, followed by restriction digestion analyses. The PCR products of NP51 and six isolates were cloned and sequenced, and BLAST search was performed. Based on these results, a species-specific primer 5'-AGTG-CAAGCACTCGGTGAT-3' for *L. animalis*, combined with a universal primer 5'-AGAGTTTGATCCTGGCT-CAG-3' targeted against variable region of 16S rRNA, was identified from the literature. This pair was used further for verification.

Results: The first primer sequence data resulted in 100% match of NP51 to *L. animalis* in BLAST search. However, the other six sequenced isolates from cattle feces matched *Escherichia coli* 536, *Escherichia coli* W3110, *Streptococcus thermophilus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Bacillus amylo-liquefaciens* in BLAST search. The second identified primers amplified NP-51 DNA but not the other six isolates, indicating this set could be used for identification of NP51 in cattle feces.

Significance: The second primer set was specific to NP51 and not to the isolates from cattle feces. Our future goals are to use these methods, to develop a q-RT PCR assay for quantification of select probiotics in environmental samples.

P4-16 An Independent Evaluation of a New Method: An Automated System for Simultaneous Detection and Differentiation of *Listeria monocytogenes* and *Listeria* Species in Food

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Introduction: Current *Listeria* screening methods involve additional testing to confirm the presence of pathogenic *L. monocytogenes*. The test simultaneously detects and differentiates *L. monocytogenes* and *Listeria* species in food and environmental samples. The enzyme-linked fluorescent immunoassay (ELFA) utilizes a sealed reagent test strip and Solid Phase Receptacle (SPR) for use on the automated system. Antigens in the sample bind to *Listeria monocytogenes* and *Listeria* antibodies coated on the inner wall of the SPR. The system cycles the sample to detect *L. monocytogenes* or *Listeria* species. The wavelength of fluoresced substrate is measured and compared to internal standards. Samples are interpreted as positive or negative.

Purpose: The purpose of this AOAC-RI independent evaluation was to compare the new method to the FDA BAM methods for produce and seafood, AOAC 993.12 for dairy and USDA-MLG for meat.

Methods: Cauliflower, fresh cod, frankfurters and vanilla ice cream were inoculated with different strains of *L. monocytogenes* (1–10 CFU/25 g). Twenty-five replicates were enriched in LX broth and incubated 24 h at 30°C. A 0.1-ml aliquot of enrichment was transferred to 6 ml of LX broth and incubated 24 h at 30°C. 1.0 ml was heated at 100°C for 5 min, added to the test strip and loaded into the system. Replicates were streaked onto chromogenic media (OAA). Typical colonies were confirmed with VITEK® *Listeria* species AOAC OMA method 992.19. Reference methods were performed as per FDA BAM, AOAC Official Methods and USDA–MLG.

Results: Of 100 samples tested, the new method gave 67 confirmed positive results compared to 65 for the reference methods. McNemar's Chi-square analysis demonstrated no significant difference between the LDUO and reference methods for all four matrices.

Significance: The new method saves critical time by detecting *L. monocytogenes* and *Listeria* species concurrently in food and environmental samples.

P4-17 Novel Phage Ligand Enzyme Linked Fluorescent Assay (ELFA) for Same Day Detection of *Escherichia coli* O157:H7 in Environmental and Feed Samples

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Introduction: Cattle and other ruminants are the most important reservoir of *Escherichia coli* O157:H7, and their environments pose a continued risk of human exposure. Moreover, these environments, i.e., the soil, the feed, the drinking water, all contaminated by fecal samples, are the main sources of *E. coli* O157:H7 infection of cattle on the farm.

Purpose: This study was designed to compare the sensitivity of the phage ELFA with a RT-PCR kit for detecting low contaminations of *E. coli* O157:H7 in environmental and feed samples, including soil, cow pad, hay and oil cake.

Methods: Five different *E. coli* O157:H7 strains were tested for each sample matrix in triplicate, with contamination levels of 1 to 5 CFU/sample. Enrichment broths (EB) were those recommended by kit manufacturers. Enrichments were incubated at 41.5°C and three time points were tested for each of the assays: 6 h, 8 h and 24 h.

Results: The kinetic studies showed the shortest incubation time that allowed optimum detection. Cow pad, feed (hay and oil cake) and soil samples proved to be positive by the phage ELFA assay only after 6 h of enrichment. The RT-PCR method rarely detected the pathogen from the cow pads, even after 18–24 h of enrichment, and was not able to give any positive result with the inoculated soil and oil cake samples.

Significance: The highly efficient host recognizing mechanisms of the phage ligand ELFA is a valuable tool for detection of low level contamination of *E. coli* O157:H7 in environmental and feed samples.

P4-18 Novel Phage Ligand Enzyme-Linked Fluorescent Assay for Same Day Detection of *Escherichia coli* O157:H7 in Composite Samples of Raw Ground Beef and Trimmings

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Introduction: Most detection kits for *Escherichia coli* O157:H7 are validated for 25 g sample sizes, which may not provide statistically meaningful results for large batches of meat samples contaminated at very low levels. The testing of 375 g composite samples has been recommended as a possible solution to this problem.

Purpose: This study was designed to compare the sensitivity of a novel phage ligand Enzyme-Linked Fluorescent Assay (ELFA) with an RT-PCR method to determine the shortest possible enrichment time for a positive result for three different samples sizes.

Methods: Five different *E. coli* O157:H7 strains were tested for each sample size in triplicate, with contamination between 1 and 5 CFU per sample size.

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Enrichment broths (EB) were those recommended by kit manufacturers, with the following sample to broth ratios : 25 g sample in 225 ml EB, 75 g: in 225 ml of EB and 375 g: in 1,125 ml of EB. Enrichments were incubated at 41.5°C and five time points were tested for each of the assays: 5 h, 6 h, 7 h, 8 h and 24 h.

Results: The kinetic studies showed the shortest incubation time that allowed optimum detection. For 25 and 75 g samples, a 6 h enrichment was sufficient, and for 375 g, an 8 h enrichment was necessary to detect all inoculated samples for the phage ELFA. The RT-PCR method was able to detect all inoculated samples at 25 g at 8 h enrichment but not at 75 and 375 g, even after 24 h.

Significance: The highly efficient host recognizing mechanisms of the phage ligand ELFA is a valuable tool for detection of low level contamination of *E. coli* O157:H7 in composite raw ground beef and trimmings up to 375 g. Composite sampling, which improves pathogen risk management, coupled to same day product release has obvious economic advantages for the meat industry.

P4-19 Surface Area and Volume Measurement of Salad and Roma Tomatoes for Microbial Enumeration

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Introduction: The microbial qualities of food surfaces are usually determined with a rinse or a sponging or swabbing procedure. Isolated and enumerated microorganisms are generally reported as counts per ml of rinse or counts per surface area. If a statistical relationship exists between the surface area of a raw fruit or vegetable and the weight of the same unit, then concentrations of organisms per surface area may be reported and compared, even though the surface was not directly measured.

Purpose: The highest concentrations of microorganisms are often found on the outer surfaces of raw foods rather than the interior. In these cases, microbial analyses may be more relevant if the complete surface of the food is sampled. The purpose of this research was to demonstrate how the surface of raw produce can be sampled and microbiological recovery reported on a concentration per unit surface area basis.

Methods: An optical imaging system was used to measure and calculate the surface area and volume of salad tomatoes and roma tomatoes. For additional tomato samples, concentrations of microorganisms recovered from surface inoculations were reported on a per volume diluent and per surface area basis.

Results: Simple linear regression showed good statistical correlation between surface area and unit weight, and high correlation between volume measurements and volume estimation from buoyant force measurement. From the regression analysis, the following equations were developed to predict surface area from weight measurements: of salad tomatoes, surface area (cm^2) = 58.7 + 0.58 Weight (g); and of roma tomatoes, surface area (cm^2) = 37.8 + 0.75 Weight (g). We observed a high correlation between concentrations reported as CFU/ ml versus CFU/ predicted sq. cm.

Significance: Our research indicates that weight measurement for surface area prediction coupled with microbiological surface sampling can be used as an alternative analytical technique to determine pathogen concentrations on some produce.

P4-20 Determination of Fumonisins B1 and B2 in Agricultural Products by High Performance Liquid Chromatography

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Introduction: Fusarium moniliforme and *Fusarium proliferatum* produce life-threatening mycotoxins called fumonisins, which have been detected in various agricultural products.

Purpose: To measure concentrations of fumonisins B1 (FB1) and B2 (FB2) in various agricultural products, this study optimized and validated a method using high performance liquid chromatography (HPLC)-fluorescence detector.

Methods: Samples were extracted by 80% MeOH and cleaned with immunoaffinity columns to prepare fumonisin fractions. The fractions were then analyzed by HPLC system (mobile phase: MeOH:0.1M NaH_2PO_4 [77:23, v/v]; excitation wavelength: 335 nm; emission wavelength: 440 nm; flow rate: 1.0 ml/min). For validation of this detection procedure, barley tea, corn flour and beer were artificially contaminated with FB1 and FB2 at 50, 100 and 200 ng/g, and the mycotoxin levels were determined by the HPLC procedure to evaluate precision and accuracy of the method, and specificity, detection (LOD) and quantification limit (LOQ) were determined on chromatograms of FB1 and FB2. In addition, linearity (R2) of the standard curve (0.05, 0.1, 0.5, 1, 5 µg/g) was calculated. The validated method was then used for monitoring of fumonisins in 156 samples (polished rice, unpolished rice, barley, barley tea, beer, sorghum, wheat flour, dried corn, popcorn, corn flour, corn tea, canned corn, breakfast cereal).

Results: Linearity (r^2) of the standard curve were 0.99998 (FB1) and 0.99995 (FB2). Precision (standard deviation) of the detection method was 2.35–4.79, and accuracy (81.47–108.83%) of analysis was also in the acceptable range (60–120%). The LOD and LOQ were 25 ng/g and 37 ng/g, respectively. From monitoring, eight samples (dried corn: 3, corn flour: 5) were FB1 positive at concentrations of 121.98-268.12 (dried corn), and 90.89-439.67 ng/g (corn flour).

Significance: These results indicate that the detection method may be appropriate for monitoring fumonisins in agricultural products. Moreover, since fumonisins were detected in corn and corn-based products, continuous monitoring for fumonisins in the products is necessary.

P4-21 Rapid Detection of Meat Freshness with Fourier Transform Infrared Spectroscopy

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Introduction: Freshness and safety of muscle foods are generally considered the most important parameters for an industry to notice. It is therefore necessary to validate and establish new rapid methods for accurate detection of microbial spoilage of meats. Fourier Transform Infrared (FTIR) spectroscopy is a rapid, non-destructive analytical technique with considerable potential for application in the food and related industries. FTIR has been tested for several muscle food analyses and recent studies on meat tissues

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stored at ambient temperature have correlated microbial spoilage of meat with biochemical changes within the meat substrate.

Purpose: The purpose of this study was to evaluate the efficacy of the FTIR technique in detecting meat freshness.

Methods: Beef fillets were stored aerobically at five different temperatures (0, 5, 10, 15 and 20°C) and microbiological analysis (Total Viable Counts) was performed in parallel with FTIR and sensory analysis. The spectral data collected from the FTIR were subjected to a principal component analysis (PCA) to investigate differences between samples and thus reduce the size of the data set. A second PCA with the selected variables (wavenumbers) revealed the Principal Components (PCs) that significantly contributed to the variance of the data set. These PCs were further subjected to factorial discriminant analysis (FDA) in order to predict the quality of a sample that was pre-characterized as Fresh (F), Semifresh (SF) or Spoiled (S) from the sensory analysis.

Results: The FDA exhibited a correct classification of 98.68%, while the cross validated FDA provided 81.58% correct classification, showing a good correlation between the sensory detection of the spoilage status and detection of chemical metabolites during spoilage by the FTIR. On the other hand, sensory evaluation of spoilage was not always correlated with the same microbial load but depended on the temperature. More specifically, the products' microbial load at the time of the first sensorial detection of spoilage (meat characterized as SF) increased with the temperature (e.g. 4.01 log CFU cm^{-2} for 0°C and 7.17 log CFU cm^{-2} for 20°C).

Significance: These results reveal that FTIR is a promising rapid tool for monitoring the freshness of meat. Complementary studies are needed to enhance the technique so it can be applied on every step of the 'farm to fork' chain.

P4-22 Rapid Discrimination of Non-O157 STEC Strains by Fourier Transform Infrared Spectroscopy

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) of various serotypes have been linked to diseases such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). *E. coli* that possess the O157 antigen are the most commonly isolated STEC worldwide; however, other serotypes such as O26, O103, O111 and O145 have also been identified as agents of foodborne illness. Fourier Transform Infrared Spectroscopy (FTIR) has been used to characterize different microorganisms based on unique spectral features of cellular constituents such as lipopolysaccharides, proteins, phospholipids and nucleic acids.

Purpose: This study was designed to evaluate the use of FTIR to discriminate non-O157 STEC strains.

Methods: Seven non-O157 strains, O145, O103:H3, O103:H10, O103:H18, O26:H11, O26:NM, and O111, were used. These strains were compared to O157:H7 ATCC 43895. Three experiments were conducted based on growth of individual strains (a) on brain heart infusion agar (BHI), (b) in meat slurry and in apple juice stored at 4°C for 6 days, and (c) under heat stress and acid stress.

Results: Characteristic absorbance peaks were observed at wavelengths between 1600 and 800 cm^{-1}. The peaks were of different shapes and sizes for both bacterial strain and growth conditions. The majority of the differences were observed at the polyglucoside peak.

Significance: These results suggest that FTIR can be a rapid tool to discriminate within the selected non-O157 STEC strains under various conditions.

P4-23 Nano-Immunomagnetic Separation of *Listeria monocytogenes*

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Introduction: It is critical to separate targets from a sample in the rapid detection of foodborne pathogens. Magnetic microbeads based immunoseparation has been practiced but still has its limitations, such as low capture efficiency and the time it requires.

Purpose: The objective of this research was to determine the efficacy of magnetic immuno-nanobeads with different sizes in the separation of *L. monocytogenes* in comparison with magnetic immuno-microbeads.

Methods: Magnetic immuno-nanobeads with a diameter of 30 nm and 150 nm were prepared by immobilizing biotin-labeled polyclonal rabbit anti–*L. monocytogenes* antibodies onto streptavidin-coated magnetic nanobeads. Then, the magnetic immuno-nanobeads were first mixed with *L. monocytogenes* and incubated for 60 to 90 min to form the magnetic immunobeads-antibody–*L. monocytogenes* complexes. The complexes were separated with a magnetic field, washed, and spread onto the MOX/TSA plates for microbial enumeration. The capture efficiency was calculated as the number of *L. monocytogenes* cells bound to the immuno-beads divided by the number of total *L. monocytogenes* in a sample. The same procedure was followed for the magnetic microbeads with a diameter of 2.8 µm for comparision.

Results: The results showed that under the same Fe concentration or surface area, 30 nm magnetic immuno-beads provided higher capture efficiency in the separation of *L. monocytogenes* than the other two immunobeads with larger diameters. The key parameters for 30 nm nanobeads, including the nanobead concentration and immunoreaction time, were optimized with different concentrations of *L. monocytogenes*. The capture efficiency increased from 36% to 82% and 94% for 60 and 90 min incubation times, respectively, as the nanobead concentration (1 mg Fe/ml) was increased from 6 µL to 50 µL when 3 log units of *L. monocytogenes* cells were used. The specificity test indicated that 30 nm magnetic immuno-beads have a low non-specific binding with *E. coli* O157:H7 and *Salmonella*, but have a 29% crosslink reaction with *Staphylococcus aureus* due to antibody non-specific reaction. The result also showed that the capture efficiency decreased from 82% to 34% when the concentration of *L. monocytogenes* was reduced from 3 log to 1 log with a 60 min immunoreaction time, but the capture efficiency could be improved significantly to 84% when the immunoreaction time was extended to 90 min. It is speculated that the surface of *Listeria* cells at low concentrations was covered with too many superfluous immuno-nanobeads, causing cell injury which then resulting in less growth on the plates.

Significance: This nano-immunomagnetic separation method could provide higher capture efficiency for *L. monocytogenes*, which would lead to more sensitive and more reliable detection of target pathogens in foods.

P4-24 DSC Sensitive Detection of *Listeria monocytogenes* Using an Impedance Immunosensor Combined with Semiconductive Nanowire Bundle

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Introduction: Nanowires/nanotubes have attracted much attention recently, especially in biosensing technologies. The combination of biosensors and nanomaterials provide the technologies for not only improving detection limit and time, but also miniaturizing the sensor for online or in field use.

Purpose: In the present study, an example was demonstrated for rapid, specific and more sensitive detection of foodborne bacterial pathogens by combining an impedance immunosensor with a semiconductive TiO_2 nanowire bundle. *Listeria monocytogenes*, which is responsible for serious infections in immunocompromised individuals and pregnant women, was chosen as a model pathogen.

Methods: A TiO_2 nanowire bundle was prepared through a hydrothermal reaction of alkali with TiO_2 powder and connected to gold microelectrodes, using a mask welding process. The gold connector surface was then blocked with 2-methyl-2-propanethiol. Monoclonal antibodies (mAbs) specific for *L. monocytogenes* were employed as capture antibodies immobilized on the surface of the TiO_2 nanowire bundle to capture target bacteria, which resulted in an impedance change. Since the antibody was vital in successful development of the immunosensor/immunoassay, detailed study was done to determine the mAbs reactivity with *L. monocytogenes*. Indirect ELISA and then transmission electron microscopy with immunogold labeling were conducted to quantitatively characterize the mAbs.

Results: The developed impedance immunosensor combined with a semiconductive TiO_2 nanowire bundle was demonstrated to be able to detect target *L. monocytogenes* at a concentration as low as 40 CFU/ml, and the total detection time was only 50 min. No significant interference was observed from non-target foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Staphylococcus aureus*. The performance of the biosensor can be further improved by optimizing the parameters of design and operation and controlling the quality of nanowire bundles.

Significance: Based on the results of sensitivity and specificity tests, the biosensor will serve as a rapid detection tool for biosafety and biosecurity.

P4-25 Impact of Variability in O157 Antigen Expression on Immuno-Capture of *Escherichia coli* O157:H7 in Beef Enrichments

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Introduction: Reliable detection of *E. coli* O157:H7 is of critical importance to the food industry in order to prevent recalls and food linked outbreaks. Recently, use of immuno-selection with anti-O157 antibodies prior to plating on selective chromogenic media for detection of this pathogen has been widely instituted. Levels of expression of this antigen can vary widely among *E. coli* O157 isolates, and little is known about regulation of its level on the cell surface. Although the prevalence of these low expressers is not usually addressed, one study has indicated that it may be over 1% of *E. coli* O157:H7 isolates.

Purpose: To examine the impact of O157 expression levels on the efficacy of antibody bead capture.

Methods: Uninoculated ground beef was enriched in BAX® MP media. For each experiment, the same enrichment was then aliquoted and post-enrichment spiked at various levels with replicates of multiple *E. coli* O157:H7 isolates expressing differing levels of the O157 antigen. Enrichments were then subjected to immuno-selection with anti-O157 magnetic beads. Following one wash in buffer, the beads were resuspended in 20 ul of buffer; and 10 ul was plated and 10 ul was subject to lysis and PCR-based detection of *E. coli* O157:H7.

Results: Greater than log level differences in recovery were seen among the isolates tested, with low O157 antigen expressing strains showing lower recovery after IMS than control strains.

Significance: Immuno-selection has limitations when used for the detection of *E. coli* O157:H7. In particular, use of shortened enrichments based on the assumption that antibody capture will improve sensitivity may be inappropriate.

P4-26 Application of Novel Bacteriophage Derived Binding Proteins for Specific Magnetic Separation of *Escherichia coli* O157 from Pure Culture and Food

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Introduction: In previous studies the application of recombinant bacteriophage proteins for specific binding and concentration of pathogenic bacteria in food samples has been successfully demonstrated. However, until now the use of such affinity ligands has been limited to the specific binding of Gram-positive organisms.

Purpose: The purpose of our study was to demonstrate that the application of magnetic particles with *Escherichia coli* O157 specific phage derived binding protein on pure culture and food samples such as ground beef, lettuce and fresh herbs would allow an efficient capture and concentration of target cells even at low contamination levels.

Methods: The developed separation method based on magnetic particles and specific binding protein was used to capture and concentrate *Escherichia coli* O157 from pure culture and artificially spiked food enrichments. The samples were subsequently plated on selective culture media (CT-SMAC) and recovery rates were determined by cultural plating in parallel. The initial inoculation rates were approximately 1 CFU/25 g, yielding fractional positive results.

Results: The results of applying the magnetic particle based capture assay on pure culture, ground beef, lettuce and fresh herbs revealed capture efficiencies in the range of 90–100%. Our results showed that this could be obtained also with very low initial cell concentration in the fractional positive range. Furthermore, following bead capture the plates showed less background flora than the plates from the reference samples, which made isolation and identification of the target organisms easier.

Significance: Because of its specificity and high capture efficiency, this magnetic particle based capture assay for *Escherichia coli* O157 represents a promising new method for concentration and purification of target organisms, removing accompanying substances, organisms and particles which otherwise could interfere with an end-point detection.

P4-27 Isolation of *Vibrio vulnificus* from Oyster Homogenate by Immunomagnetic Separation Using Anti-H Monoclonal Antibodies

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Introduction: *Vibrio vulnificus* is cited as a sole cause of about 350 illnesses per year in the United States, with an overall 40% mortality rate. Conventional methods for the detection and enumeration of *V. vunificus* from environmental samples are labor-intensive and time-consuming. Immunomagnetic beads (IMB) coated with monoclonal antibody specific for *V. vunificus* could prove useful for the isolation and concentration of the organism from complex environmental samples.

Purpose: This study was aimed at developing an immunomagnetic separation protocol using anti-H monoclonal antibody for the recovery of *V. vunificus* from spiked oyster homogenates.

Methods: Two different sets of IMB were prepared by mixing sheep anti-mouse IgG IMB with monoclonal antibodies reactive with *V. vunificus* flagellar core at a concentration of 5 µg IgG/107 IMB. The binding capacity of the beads coated with monoclonal antibodies was determined by incubating 10^7 IMB with 500 µl spiked oyster homogenate on a shaker at 25°C for 30 min, followed by separation with an immunomagnetic bead concentrator. The number of unbound bacteria was determined by plating the aspirated supernatant fluid on TCBS and TSA plus 2% NaCl agar plates.

Results: Two clinical strains of *V. vulnificus* at two different concentrations, $1–5 \times 10^2$ and $1–6 \times 10^3$ *V. vulnificus*/ml, respectively, exhibited binding capacities up to 35% and 78% dependent on cell concentration and monoclonal antibody employed.

Significance: This technique could lead to a simple and rapid method for oyster risk assessment.

P4-28 DSC Development of a Real-Time, NASBA-Molecular Beacon System for Rapid and Specific Detection of Live Microbes in Juice Products

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Introduction: Food spoilage due to the outgrowth of spoilage microorganisms causes significant financial loss to the industry. Molds and yeasts are particularly problematic due to their abilities to survive and grow at a wide range of pH and water activity. Conventional detection methods are time-consuming. Rapid, specific and sensitive detection of the slow-growing live fungal cells is needed for proper food quality control.

Purpose: The purpose of this project is to develop a real-time, isothermal Nucleic Acid Sequence-Based Amplification (NASBA)-Molecular Beacon system to detect the presence of live yeasts in juice products.

Methods: RNA molecules are abundant only in live cells, and therefore were targeted for detection. A pair of NASBA primers and a molecular beacon probe targeting conserved areas of the 18S rRNA gene were designed and synthesized. The RNA template was extracted and subjected to DNase treatment at 41°C before the NASBA. Selected microorganisms commonly found in the food environment and common fruit ingredients were used in the detection specificity analysis. The sensitivity of the detection method was determined by comparing positive NASBA results with conventional plate counting methods using serially diluted yeast cells.

Results: Using the above primers-and-probe set, less than 1000 *Saccharomyces cerevisiae* and *Candida parapsilosis* cells in juice were detected without cross-activity to molds, bacteria and raw food materials by the real-time NASBA-Molecular Beacon system. The analysis was completed within 8 h. No NASBA signal was detected for autoclaved yeast samples.

Significance: The results suggest that the developed real-time NASBA-Molecular Beacon system can be used to rapidly and specifically detect live spoilage yeasts in liquid food. Application of the developed detection system can enhance quality control and minimize spoilage in juice products.

P4-29 Practicality and Validity of Protein-Wiping Method of Sanitation Self Inspection in Food-Processing Plants

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Introduction: Many efforts have been made for microbial control in the food-manufacturing industries. Simple and rapid detection methods are needed to monitor the hygienic environment in the food-processing plants. Therefore, low initial costs and low running costs of detection methods have been sought by the food manufacturers. In this study, three different detection methods for microbial control in actual food processing plant, including protein-wiping test, microbial test, and visual observation testing by the site foreman, were performed. The validity of the protein-wiping inspection was compared to the other two detection methods.

Purpose: The purpose of this study was to evaluate the protein-wiping test for monitoring the hygienic environment in actual food-processing plants.

Methods: The practical application and validation of the protein-wiping method of self-inspection in food-processing plants were done. Three different ways of detecting contamination in actual food-processing lines (producing omelets, Japanese style confectionery, chicken, side-dishes, vegetables, etc.), protein-wiping inspection, microbial inspection, and visual observation testing by the site foreman, were tested. The relationship among the results of the protein-wiping method, microbial contamination testing (expressed as total viable number and coliform number), and observation of food residues was investigated.

Results: Detection limit of the protein-wiping method was evaluated to be within 40 to 80 µg of protein. One hundred fifty-two samples were tested in total; the coincidence rate between the results of the protein-wiping inspection and those of the other two methods was 94.7%. Thus, the results of the protein-wiping method strongly reflected microbial contamination. Only three samples (2.0%) were found false negative; however, microbial contamination levels in these samples were relatively low (less than 10^2 CFU/100 cm^2).

Significance: The present study demonstrated that the protein-wiping method is a simple and rapid inspection means for detecting microbial contamination in food-processing plants which does not require special skills.

P4-30 Rapid Tools for Microbial Forensics in the Food Industry

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Introduction: Rapid bacterial identification and characterization plays a major role in developing intervention strategies in the food industry to both control food spoilage and to maintain food safety of products. The advent of molecular based techniques such as ribotyping and DNA sequencing has reduced the time to result and of these, microbial DNA sequencing is considered to be the gold standard for microbial identification. Molecular techniques have several benefits, including the independence on microbial growth or protein expression for successful test completion, and a reduction in subjectivity when interpreting results.

Purpose: This study will focus on sequence analysis and repeat based PCR for microbial analysis, using specific examples isolated from a range of food products.

Methods: The research used the Microseq® (Applied Biosystems) and DiversiLab (bioMérieux) systems to identify unknown bacterial and fungal isolates and to characterize a panel of microbial isolates. Two distinct case studies using the salmonellae and over 20 isolates of *Penicillium* spp. (and other closely related spp.) were analyzed. All case studies were done in accordance with the manufacturer's instructions.

Results: Two selected case studies using the Microseq® system revealed the potential for sequence analysis to provide same day (6-h turnaround) answers to bacterial and yeast contamination issues. Analysis of the data from the DiversiLab rep PCR (characterization) work demonstrated the potential of the technology to rapidly discriminate between isolates and to track sources of contamination in food-related environments.

Significance: The case studies presented demonstrate the power and flexibility of molecular techniques to provide rapid answers to bacterial, yeast and mold contamination issues.

P4-31 Rapid Confirmation and Characterization of Food Related Salmonellae

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Introduction: The foodborne pathogen *Salmonella enterica* has been isolated from a wide range of food types and has been associated with a number of outbreaks worldwide. Rapid confirmation and characterization of the salmonellae is critical in the effective and timely management of contamination issues, to minimize the impact on consumer safety and the loss of brand confidence. Recent developments in technology such as PCR and nucleic acid probes have reduced the time to result for confirmation and characterization, to enable same day reporting of results.

Purpose: The aim of the current study was to evaluate the use of commercially available assays to rapidly confirm and characterize the salmonellae.

Methods: A selection of four assays (AB TaqMan *Salmonella enterica* detection kit, Qualicon BAX *Salmonella* kit, REMEL RapID ONE, Sy lab *Salmonella* RNAssay) for *Salmonella* confirmation were tested, using a panel of 50 *Salmonella* isolates and 20 non-*Salmonella* isolates. Overnight cultures streaked onto nutrient agar were tested according to the manufacturer's instructions. In addition to the confirmation work, a selection of the panel were also characterized using the repeat based PCR (rep PCR) Diversilab *Salmonella* kit as detailed in the recommended protocol.

Results: Data from the confirmation study showed that all four methods were able to confirm over 98% of the *Salmonella* isolates. Characterization of the *Salmonella* isolates revealed that the technique is able to discriminate between serotypes of salmonellae.

Significance: The results from the two studies revealed the potential for rapid technologies to provide timely answers to assist in making key commercial decisions.

P4-32 Effect of Salt and Acid and the Sequence of Application on the Growth Boundaries of *Escherichia coli*

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Introduction: Many food products are preserved by using a combination of preservative factors that are not lethal by themselves but that together are sufficient to disrupt microbial growth. Salt, acid and chemical preservatives form the basis of this approach and these tend to be added to the product as bulk ingredients during formulation in the factory. However, research has suggested that instead of multiple stresses achieving better inhibition of microorganisms, the use of one stress factor may in fact confer resistance to a range of other environmental stresses. This aim of this project was to investigate the response of microorganisms to different stresses in order to ensure that food manufacturing procedures are optimized in terms of preservation.

Purpose: To determine the effect of salt and pH stress, applied either together or in sequence, on the growth and survival characteristics of *Escherichia coli.*

Methods: Two strains of *E. coli* (K12: CRA 1881 and NCTC 12441) were grown in Nutrient Broth under combinations of pH (7.0 to 4.2) and NaCl (1 to 8%) to establish the boundary of growth/no growth. Combinations of pH and NaCl which were inhibitory when applied simultaneously were investigated further. At the beginning of the growth experiments, only one of the stresses was present, e.g., pH. The broths were incubated at 30°C and growth allowed to initiate. After a 4-h delay, the second stress was applied to the broths, e.g., NaCl, which were then reincubated and the subsequent growth and survival characteristics determined.

Results: The subsequent growth data showed that there were differences in effect on the growth and survival of *E. coli,* depending on whether salt or pH was applied as the second stress. Where salt was applied as the second stress, there was an immediate cessation in growth followed by cell death. However, where pH was applied as the second stress, there was a slight reduction in cell numbers, but growth resumed after a period of adaptation.

Significance: This work has shown that there is a marked effect on the survival characteristics of *E. coli* around the growth boundary, depending on sequence of application of stress factors. When pH and salt are together at the start of the trial or pH is present and salt is added after 4 h, no growth is observed. However, if salt is present at the start of the trial and pH is changed after 4 h, then growth occurs. This demonstrates the principles of hurdle technology, where the application

of multiple stresses may not always lead to enhanced preservation if one stress can increase resistance to subsequent stresses.

P4-33 Reduction of *Escherichia coli* O157:H7 on Lettuce Using Electrolyzed Oxidizing Water under Simulated Food Service Operation Conditions

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Introduction: Outbreaks of *Escherichia coli* O157:H7 infections associated with fresh produce have been documented. Treatment of produce with electrolyzed oxidizing (EO) water has been shown to kill or reduce foodborne pathogens attached to the surface of produce.

Purpose: The objective of this study was to evaluate the efficacy of EO water in killing *E. coli* O157:H7 attached to iceberg lettuce. Conditions for treatment simulated those that may exist in salad preparation areas in food service kitchens.

Methods: Whole lettuce leaves were spot inoculated with a mixture of five *E. coli* O157:H7 strains (8 log CFU/leaf), dried at 21°C for 2 h, and held at 4°C for 20–22 h. Running acidic EO water (14 A, 40 µg/ml free chlorine) or running tap water (control) at 22 ± 2°C was used to wash the inoculated leaves for 30 s. In a second experiment, chilled (4°C) acidic EO water (16 A, 38 µg/ml free chlorine) or tap water was used to quick-chill cut lettuce for 15 min.

Results: Washing lettuce leaves with running EO or tap water reduced populations of the pathogen up to 1.8 and 1.2 log CFU/leaf, respectively. Quick chilling lettuce with tap water removed up to 0.6 log CFU/cut leaf, while treatment with 16 A EO water reduced the pathogen up to 1.3 log CFU/cut leaf.

Significance: Results indicate that application of EO water using a process mimicking that of a restaurant or food service operation can reduce the risk of *E. coli* O157:H7 being present on iceberg lettuce at the time of consumption.

P4-34 Antibiotic Testing of Enterohemorrhagic *Escherichia coli* Isolated from Ground Beef Samples by Use of a Spiral Gradient Endpoint Method

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is one of the most important recently emerged group of foodborne pathogens. Ground beef is often implicated in foodborne outbreaks of *E. coli* O157:H7. Antibiotic usage is possibly the most significant factor that promotes the emergence, selection and propagation of antibiotic-resistant microorganisms.

Purpose: The objective of the present study was to determine the antibiotic susceptibility patterns of verotoxin-producing *Escherichia coli* strains isolated from ground beef.

Methods: Thirty-nine EHEC strains isolated from ground beef samples were analyzed for antimicrobial susceptibilities to 13 antibiotics, using the Spiral Gradient Endpoint (SGE) method to determine the end concentration (EC), tail end concentration (TEC) and gradient minimum inhibitory concentration (GMIC) at a breakpoint of 0.5–512 µg/ml.

Results: Two isolates (5%) were resistant to at least four antibiotics, while 37 (95%) of the strains were resistant to five to nine antibiotics. These isolates were observed to be resistant to sulfamethoxazole, sulphadimidine, tylosin tartrate (GMIC = ≥ 256 µg/ml; EC = ≥ 145 -178 µg/ml; TEC = ≥ 512 µg/ml) and tetracycline (GMIC = 8 µg/ml; EC = 5.4 µg/ml; TEC=11.3 µg/ml) while all (100%) of the isolates were susceptible to trimethoprim, ciprofloxacin, enrofloxacin, norfloxacin and ofloxacin (GMIC = ≤1 µg/ml; EC = ≤ 0.8 µg/ml; TEC = ≤ 0.8 µg/ml). Overall, the class of antibiotics with the highest number of resistant strains was the folate inhibitors, compared to the fluroroquinolones, which exhibited the highest number of susceptible EHEC strains.

Significance: Periodic surveillance of antibiotic susceptibilities may be an important measure in detecting emergence and spread of resistance. Antibiotic susceptibility testing may be a useful tool for typing strains of foodborne pathogens.

P4-35 DSC Attachment and Growth of *Escherichia coli* O157:H7 on Stainless Steel as Affected by Nutrient Level, Ground Beef Residues and Natural Flora

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Introduction: Escherichia coli O157:H7 may attach and grow on beef residues on stainless steel equipment surfaces and subsequently contaminate additional product. It would be useful for better selection of sanitation programs to evaluate the level and strength of attachment (Sr) of *E. coli* O157:H7 on stainless steel under various conditions.

Purpose: This study evaluated the effects of fluid flow, beef-grinding residues, nutrient density, natural flora, and time on the level and strength of attachment and growth of *E. coli* O157:H7 on stainless steel.

Methods: Stainless steel coupons (2 × 5 cm) inoculated by submersion or with constant volume of culture of rifampicin-resistant *E. coli* O157:H7 (5 strains; 3 log CFU/cm²) were incubated (4 days) at 15°C in 10-fold diluted Tryptic Soy Broth or beef-grinding residues (filter-sterilized or unsterilized) either under agitation (60 rpm) or statically. Loosely and firmly attached *E. coli* O157:H7 and total bacterial cells were removed with sequential vortexing and enumerated, along with planktonic cells, on Tryptic Soy Agar (TSA) with rifampicin (100 µg/ml) and TSA, respectively, and used to calculate Sr. Two complete replicates were performed, using three samples in each, and statistical analysis of the data was performed using the PROC GLM procedure of SAS.

Results: Counts of attached (3.0 log CFU/cm²) total microbial flora increased to 6.0–7.0 log CFU/cm² with time in all substrates, while those of *E. coli* O157:H7 decreased to < 2 log CFU/cm² in unsterilized beef-grinding residues ($P < 0.05$). Planktonic *E. coli* O157:H7 cell counts in unsterilized beef-grinding residues were higher ($P < 0.05$) than those attached, but still lower than total flora and *E. coli* O157:H7 in other substrates. The initial Sr was low (0.04–0.06) and increased only in beef-grinding residues (0.34 ± 0.09).

Significance: These data suggest that, on stainless steel surfaces present in beef processing, although ground beef residues may provide an environment for stronger attachment, natural flora may outgrow *E. coli* O157:H7.

P4-36 DSC Cross Protection of Acid-Adapted *Escherichia coli* O157:H7 against Activated Lactoperoxidase and Low pH

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Introduction: The capacity of *Escherichia coli* O157:H7 to survive in acidic foods is attributed to its ability to adapt to acidic conditions that confer acid-resistance to a lethal acid environment and subsequent cross protection against other unrelated stresses. Cross-protection studies with acid-adapted *E. coli* O157:H7 in combination with natural preservation interventions such as lactoperoxidase (LP) need to be investigated in order to develop methods to effectively control their presence in foods.

Purpose: The objective of this study was to determine whether acid-adapted *E. coli* O157:H7 elicits cross-protection against the LP system in combination with low pH in Tryptic Soy Broth (TSB).

Methods: E. coli cells were acid-adapted by growing them in TSB supplemented with 1% glucose while non-adapted cells were grown in TSB supplemented with 100 mM MOPS for 18 h at 37°C. Acid-adapted and non-adapted cells were challenged in TSB acidified with lactic acid to pH 4, 5 or 7 in combination with activated LP (10 µg/ml) for 24 h at 25°C and enumerated on Tryptic Soy Agar. Outer membrane proteins (OMPs) and fatty acids (OMFAs) of challenged cells were determined by gas chromatography and polyacrylamide gel electrophoresis, respectively.

Results: There was an overall significant ($P = 0.000$) difference between survival of acid-adapted and non-adapted cells challenged with the LP system in combination with pH 4, 5 and 7. Acid-adapted cells elicited cross-protection by showing less than 1 $\log_{10}$ reduction while non-adapted cells were reduced to undetectable levels (detection limit of 10 CFU/ml) after a 24h challenge to LP at pH 4. Increase in saturation of OMFAs and higher intensity band of OmpC in acid-adapted cells compared to non-adapted cells contributed to cross protection.

Significance: Cross protection of acid-adapted *E. coli* O157:H7 poses a threat to food safety and should be considered in designing preservation tools to prevent their occurrence in milk and fermented milk products.

P4-37 Survival and Growth of Acid Adapted *Escherichia coli* O157:H7 in Traditional Goat Milk Amasi

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Introduction: Fermented foods, which have been regarded as safe, have been implicated in the outbreak of foodborne diseases caused by *Escherichia coli* O157:H7. The outbreaks are attributed to adaptation of microorganisms to low pH.

Purpose: The purpose of this study is to determine the growth and survival of acid adapted *E. coli* O157:H7 in traditional and modern goat milk amasi.

Methods: Traditional and modern goat milk amasi was inoculated with acid adapted (AA) or non-adapted (NA) *E. coli* O157:H7 and survival was determined during fermentation at ambient temperature or fermentation for 24 h at ambient temperature and storage at 7°C for 3 days.

Results: E. coli O157:H7 counts in traditional amasi inoculated at pH 5.6 were significantly different ($P < 0.05$) from those of modern amasi. *E. coli* O157:H7 counts in modern amasi were detected at 2.7 log CFU/ml after 3 days, while those in traditional amasi could not be detected after the same period. There was no significant difference ($P < 0.05$) in the survival of AA and NA *E. coli* O157:H7 in traditional amasi inoculated at pH 5.6, while in modern amasi, inoculated at pH 5.6 or after pasteurization, the NA strain survived significantly ($P < 0.05$) better than its AA counterpart. Acid-adapted *E. coli* O157:H7 could not be detected after 24 h when inoculated after pasteurization, while the NA strain could be detected at 4.2 log CFU/ml after the same period.

Significance: The results indicate that *E. coli* O157:H7 can survive in modern amasi up to the point of consumption, but that the natural bacterial composition of traditional amasi seems to affect the growth of both NA and AA *E. coli* O157:H7.

P4-38 Increased Acid Resistance of Acid-Adapted *Escherichia coli* O157:H7 Isolated from Different Sources in Acetic Acid Solution

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Introduction: A number of studies on the influence of acid on *E. coli* O157:H7 have shown considerable strain differences, but limited information has been reported on comparing the acid resistance based on different sources of *E. coli* O157:H7 isolates under exposure to various environmental conditions that simulate those found in foods.

Purpose: The purpose of this study was to determine the stationary-phase resistance of *E. coli* O157:H7 isolated from five different sources (food, bovine carcass, bovine feces, water, and human outbreak isolates) in acetic acid solutions exposed to different environmental conditions.

Methods: Fifty-eight strains of *E. coli* from various sources (food, bovine carcass, bovine feces, water and human outbreak isolates) were used in this study. A 200 µl cocktail of *E. coli* O157:H7 was added to 1800 µl acetic acid solutions (400 mM) with or without 2 ml glutamic acid in 12-well tissue culture plates and incubated aerobically at pH 3.3 and 30°C for 25 min; then, *E. coli* cells were immediately diluted 10-fold by adding 20 µL to 180 µL 0.1 M MOPS buffer to neutralize pH prior to plating. Genomic fingerprint was analyzed to determine genetic diversity of different strains.

Results: Human outbreak (1.39–5.59 log CFU/ml) and food isolates (1.15–5.57 log CFU/ml) were more sensitive than bovine carcass (0.71–3.85 log CFU/ml), water (0.51–3.59 log CFU/ml), and bovine feces (0.84–2.74 log CFU/ml) isolates irrespective of acid adaptation. The acid resistance of all of the different sources of isolates in acid adapted conditions was significantly increased ($P < 0.05$) compared with those in non-acid adapted conditions. The addition of glutamate to the acetic acid solution provided the best protection, with 4 of 58 isolates exhibiting a log reduction of less than 0.7. The fingerprint patterns among food, water or bovine feces group were relatively similar but more diverse genetic patterns were observed in human or bovine carcass isolates.

Significance: This study revealed that the previous history of this pathogen should be a consideration when designing microbial safety strategies for acid foods.

P4-39 DSC Role of Exopolysaccharides in Protecting the Cells of Shiga-Toxin Producing *Escherichia coli* against Chlorine Treatment

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Introduction: Exopolysaccharides, comprised of colanic acid (CA) or cellulose (Cel) are produced by some strains of Shiga-toxin producing *Escherichia coli* (STEC). Previous studies have shown that the CA-producing cells have greater tolerance to oxidative and osmotic stress. Chlorine is a sanitizing agent commonly used by the food processing industry for disinfecting equipment, utensils and water supplies, as well as carcass chilling waters.

Purpose: The goal of this study was to define the role of Cel, and Cel in conjunction with CA, in protecting the cells of STEC against chlorine treatment.

Methods: STEC cells producing solely Cel (Cel+CA-) or Cel in conjunction with CA (Cel+CA+) and their Cel-deficient mutants (Cel-CA- or Cel-CA+) were treated with 0, 25, 50, or 100 µg/ml of sodium hypochlorite at ambient temperature with gentle shaking. Samples were withdrawn at 1, 3, 5, and 10 min intervals during the treatments, and surviving STEC cells were subsequently enumerated.

Results: Results showed that Cel-proficient cells were relatively more persistent ($P < 0.05$) than their Cel-deficient counterparts. Approximately 5.13, 4.28, or 3.81 $\log_{10}$ CFU/ml of the Cel+CA- cells and none of the Cel-CA- cells (8.04 logs) survived the 5-min treatments with 25, 50 and 100 µg/ml of sodium hypochlorite, respectively. The population of Cel-CA+ cells were reduced from 7.99 to 0.58 $\log_{10}$ CFU/ml by a 10-min treatment with 100 µg/ml of sodium hypochlorite, whereas the population of Cel+CA+ cells was 2.71 $\log_{10}$ CFU/ml at the end of treatment; cells of the CA-producing (Cel-CA+ and Cel+CA+) STEC were relatively more persistent ($P < 0.05$) to treatments with 25 µg/ml of sodium hypochlorite, compared with those that did not produce CA (Cel-CA- and Cel+CA-). However, both types of cells were equally vulnerable to the treatments with over 50 µg/ml of sodium hypochlorite.

Significance: The study indicated that both Cel and Cel in conjunction with CA protected STEC cells against chlorine treatment.

P4-40 Production of Cellulose by the Cells of Shiga Toxin-Producing *Escherichia coli* as Influenced by Different Environmental Conditions

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Introduction: Cells of some STEC are capable of producing cellulose, which is known to mediate cell-cell interaction, protect cells against stress, and provide a tight and rigid structure as well as extra strength to biofilm.

Purpose: The goal of this project was to determine the optimal environmental conditions for cellulose production by STEC cells.

Methods: Six strains of STEC were grown on Luria-Bertani no salt (LBNS) agar at different temperatures and atmospheres for 96 h. The amounts of cellulose produced by the STEC cells were quantified with a colorimetric assay. In separate experiments, ethanol (0, 1 and 2%) or fructose (0, 0.4, 1 and 2%) was added to LBNS agar. Media pH values were adjusted to 5, 6 or 7, and water activities to 0.96, 0.97, 0.98 or 0.99. The STEC cultures were grown on each media at 28°C for 96 h before the produced cellulose was quantified.

Results: The average amount of cellulose produced by the six STEC strains at 28°C was significantly higher than that produced at 15, 22 or 37°C. Greater cellulose production was observed under aerobic (2.62 µg/10^{10} CFU) followed by microaerophilic (2.41 µg/10^{10} CFU) and anaerobic (1.72 µg/10^{10} CFU) conditions. Media with a water activity < 0.99 were not suitable for cellulose production. Culture media with pH 6 was most favorable for cellulose production, with only one exception. The average amounts of cellulose produced by the six STEC cultures at pH 6, 7 or 5 were 4.29, 3.59 and 2.95 µg/10^{10} CFU. The addition of ethanol significantly increased the rate of cellulose production, opposite to the addition of fructose to the growth media.

Significance: Environmental conditions optimal for cellulose production by STEC included a 28°C incubation temperature, aerobic atmosphere, and presence of 2% ethanol in LBNS agar with a pH value of 6 and water activity of 0.99.

P4-41 Prevalence of Shiga-Toxigenic *Escherichia coli* and *Salmonella* in Commercially Available Compost

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Introduction: Proper thermophilic management substantially reduces pathogen content of composts. Commercial compost quality varies by management/feedstock and is regulated by states unless it contains sewage sludge, when it must then meet federal pathogen reduction standards (40CFR Part 503). Most states do not stipulate a pathogen content standard.

Purpose: This study was conducted to characterize the microbial quality of finished, marketable compost prepared from a wide range of residuals from 15 US commercial facilities.

Methods: Samples (n = 108) of mature compost were collected in March, August and November 2000 and enumerated by MPN and spread plating techniques for fecal coliforms, *E. coli* and *Salmonella* spp. Whole cell FAME profiles of 261 *E. coli* isolates were used to select 183 isolates for virulence factor screening. Multiplex PCR assays targeting *Stx*1, *Stx*2 and *eae* genes of *E. coli* O157:H7 were conducted.

Results: Results show 53% and 6.7% of compost facilities had product that exceeded the USEPA 503 limit for Class A product: fecal coliforms > 1000 MPN/g and *Salmonella* > 3 MPN/4g, respectively. One *E. coli* isolate (0.55%) positive for *Stx*2 was recovered from sewage sludge compost that met the EPA503 standards. In total, 67% of samples were positive for *E. coli* and 78.7% of samples met the fecal coliform standard.

Significance: Prevalence of toxigenic *E. coli* in commercial composts was very low despite the relatively high number of samples that contained *E. coli*. All facilities use outdoor compost systems in which most, but not all, particles are subjected to pathogen destructive thermal process time-temperatures. Data in this study show that commercial composts that meet the fecal coliform and *Salmonella* standards may still contain low levels of pathogenic *E. coli*. Pathogen content data are needed to help ensure selection and use of quality composts by fresh produce growers.

P4-42 Characterization and Potential Human Health Risks of Shiga Toxin-Producing *Escherichia coli* from Beef Cattle on the Range

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Introduction: Human illnesses from foods contaminated with Shiga toxin-producing *Escherichia coli* (STEC) result from the toxin produced by these pathogens. These include Shiga toxin 1 (Stx_1), Shiga toxin 2 (stx_2), α-hemolysin (*hlyA*), and/or enterohemorrhagic *E. coli* [EHEC]-hemolysin (EHEC-*hlyA*). These proteins are encoded by stx_1, stx_2, *hlyA*, and EHEC-*hlyA*, respectively.

Purpose: The objective of this study was to examine presence and expression of these genes in STEC isolates of bovine origin.

Methods: A total of 77 STEC isolates were recovered from testing fecal samples (n = 774) from six cow/calf operations in California (cattle grazing rangelands) over one year.

Results: The STEC isolates belonged to 35 serotypes (O1:H2, O5:H$^-$ [nonmotile], O26:H11, O39:H$^-$, O84:H2, O84:H$^-$, O86:H2, O96:H19, O111:H16, O111:H$^-$, O116:H2, O116:H36, O125:H2, O125:H16, O125:H19, O125:H27, O125:H28, O125:H$^-$, O127:H2, O127:H19, O127:H28, O128:H2, O128:H16, O128:H20, O146:H21, O157:H7, O158:H16, O158:H19, O158:H28, O166:H2, O166:H6, O166:H20, OUT [untypeable O antigen]:H2, OUT:H19, and OUT:H$^-$) and were lethal to Vero (African green monkey kidney) cells. Of these isolates, 35, 12, and 30 had stx_1, stx_2, or both genes, respectively. Except for three (isolates belonging to the O84:H$^-$, O127:H2, and O158:H19 serotypes, having both genes, and expressing only stx_1), all isolates expressed their *stx* genes. Nine isolates (belonging to the O1:H2, O111:H16, O125:H2, O125:H16, O127:H2, and O166:H6 serotypes) had and expressed *hlyA*, 47 other isolates had EHEC-*hlyA*, and only 37 expressed it. Of the35 serotypes detected, 10 (O5:H$^-$, O26:H11, O84:H$^-$, O111:H$^-$, O125:H$^-$, O128:H2, O146:H21, O157:H7, OUT:H2, and OUT:H$^-$) are known to cause hemolytic uremic syndrome and three (O1:H2, O84:H2, and OUT:H19) are known to cause other human illnesses.

Significance: Our STEC isolates produced one (n = 14), two (n = 53), or three (n = 10) virulence factors and as a result are a health risk to humans.

P4-43 Determining the Impact of Environmental Factors on the Regrowth of *Escherichia coli* O157:H7 in Dairy Compost

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Introduction: Recently, several large produce-related outbreaks of *E. coli* O157:H7 and *Salmonella* have led to concerns about contamination of vegetables with fecal pathogenic bacteria during preharvest. Despite several studies regarding the survival of pathogens in compost, little attention has been paid to pathogen regrowth under various environmental conditions.

Purpose: The objective of this study was to investigate environmental variables such as physiological stage of inocula, strain variation, moisture, indigenous microflora and nutrients for *E. coli* O157 regrowth in dairy compost.

Methods: Three rifampin-resistant *E. coli* O157 strains grown previously in full or reduced strength media were inoculated into dairy compost (autoclaved or non-autoclaved) with 30% moisture content and stored at 22°C for 7 days. Different autoclave times (1, 2 and 3 times) and moisture contents (10, 20, 30, and 40%) on *E. coli* O157 regrowth were compared.

Results: The growth rate of *E. coli* O157 in compost was highest in the spinach-outbreak strain. All three strains regrew in autoclaved compost, ranging from 1.09 to 2.49 log CFU/g in 2 days, with the highest increase in the spinach-outbreak strain. There was no significant difference in population increase between 1 and 2 times autoclaved compost, showing approx. 4.6 log CFU/g increase in 5 days. Non-autoclaved compost with 40% moisture content did not support *E. coli* O157 regrowth, whereas repopulation occurred in autoclaved compost with 20% moisture content. The plate count data also revealed that *E. coli* O157:H7 adapted to the reduced nutrient media regrew to higher population than the control cultures.

Significance: Our results suggest that a few *E. coli* O157 cells can regrow in dairy compost for up to 6.7 log CFU/g when background microbial population is low and the moisture content of compost was maintained at 20% or higher. Therefore, contaminated compost could serve as a source of pathogen contamination of fresh produce on farms.

P4-44 Characterization and Evaluation of Aptamers Isolated against *Listeria monocytogenes*

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Introduction: Listeria monocytogenes is a pathogenic species causing listeriosis, which is a severe infectious disease characterized by meningitis and septicemia and which can cause spontaneous abortion. Because 1 cell of *L. monocytogenes* per gram of food has been associated with the disease in susceptible individuals, the development of rapid and sensitive detection methods for *L. monocytogenes* in food continue to be of importance. Aptamers have been considered alternatives to antibodies for the development of analytical and diagnostic methods.

Purpose: The purpose of this study was to characterize isolated aptamers specific for *Listeria* and evaluate their ability to capture *L. monocytogenes* on nanomagnetic beads.

Methods: Aptamers against *L. monocytogenes* were isolated with the SELEX process, using recombinant internalin A and B as targets. Competitive ELISAs using the target proteins and increasing concentrations of non-labeled aptamers were performed to determine the EC50. Capture efficiency of *L. monocytogenes* from aptamer-coupled nanomagnetic beads was tested in buffered peptone water or phosphate buffer.

Results: An aptamer (A8) against internalin A with EC50 of 543.5 nM was demonstrated to have a high capacity for binding *L. monocytogenes,* based on ELISA data. An aptamer (R32) against internalin B with EC50 of 82.7 uM demonstrated poor ability to bind *L. monocytogenes*. Capture efficiency of *L. monocytogenes* using A8 aptamer coupled nanomagnetic beads was determined to be higher (.21% ± .05%) in phosphate buffer than in buffered peptone water (.06% ± .02%).

Significance: Significance: The data for the high affinity aptamer A8 indicates its potential to be used as

a capture agent on nanomagnetic beads and for the development of a new detection assay against *L. monocytogenes* for the timely detection of *L. monocytogenes* in fresh and processed foods.

P4-45 DSC Evaluation of the Diversity and Distribution of *Listeria monocytogenes* in Retail Food Establishments, Using Pulsed-Field Gel Electrophoresis and Automated Ribotyping

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Introduction: Listeria monocytogenes is a foodborne pathogen capable of causing severe disease. Genetic characterization of *L. monocytogenes* by pulsed-field gel electrophoresis (PFGE) or automated ribotyping can aid in the identification of human listeriosis clusters and contaminated food sources.

Purpose: To evaluate *L. monocytogenes* ribotype and PFGE subtype diversity and distribution from food and environmental locations in retail establishments.

Methods: L. monocytogenes isolates from food (n = 6) and environmental sponges (n = 151) were obtained from 1351 total samples. Ribotyping was performed by use of EcoRI with the Riboprinter Microbial Characterization System (Qualicon Inc.). Ribotype patterns were assigned according to DuPont ID. PFGE was performed according to standardized Centers for Disease Control and Prevention (CDC) PulseNet protocols using AscI. PFGE types were compared using Bionumerics 4.6 (Applied Maths). Unique PFGE patterns were assigned on the basis of at least one band difference between patterns. Molecular diversity and suitability of typing methods for discrimination of strains was determined using Simpson's Index.

Results: There were 29 unique ribotypes and 80 unique PFGE types identified among 157 *L. monocytogenes* isolates, with Simpson's Index values of 0.891 (95% CI = 0.869–0.914) and 0.984 (95% CI = 0.978–0.990), respectively. Riboprinting identified 8 ribotypes occurring at least five times and 20 ribotypes occurring only once. PFGE identified 6 PFGE types occurring at least five times and 46 PFGE types occurring only once. No PFGE types were further discriminated by ribotyping, while 9 ribotypes were discriminated by at least one PFGE type. Two of three establishments from which foods and environmental samples that contained *L. monocytogenes* were obtained showed the presence of the same *L. monocytogenes* ribotype and PFGE type in food as in environmental isolates.

Significance: While automated ribotyping is a rapid and moderately discriminating subtyping method for *L. monocytogenes*, the use of a more highly discriminating method (e.g. PFGE) may improve source tracking of *L. monocytogenes* in retail establishments.

P4-46 DSC Proteome-Based Studies for Inhibition of Biofilm Formation of *Listeria monocytogenes* by β-Casein Glycomacropeptide

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Introduction: Listeria monocytogenes has the ability to attach to various surfaces and form biofilms. Several reports have shown that biofilms produced by *L. monocytogenes* are more resistant to environmental changes and cleaning treatments than are cells in the planktonic growth mode. Therefore, new inhibitors with the potential to remove mature biofilms are needed.

Purpose: Our objective was to examine the capability of β-casein glycomacropeptide (CMP) to inhibit biofilm formation by *L. monocytogenes*. This was analyzed with two dimensional electrophoresis (2-DE).

Methods: Based on various conditions, the biofilm formation of *L. monocytogenes* strains on polyvinylchloride (PVC) microtiter plates were indirectly assessed by staining with 0.1% crystal violet. On the selected strains, inhibition of biofilm formation by CMP (0.1, 0.2 or 0.4 mg/ml) was determined by the above methods. In addition, the inhibition factors of CMP were also analyzed by 2-DE.

Results: In the PVC microtiter plate assays, 12 strains of *L. monocytogenes* exhibited biofilm formation. The inhibition rate of *L. monocytogenes* strains biofilm formation was the lowest in Modified Welsheimer's Broth (MWB) containing 0.4 mg/ml of CMP. Five proteins exhibited lower levels of expression in MWB containing 0.4 mg/ml of CMP.

Significance: This property of CMP could contribute to understanding specific mechanisms within bacterial communities and lead to the development of novel and food-grade adjuncts for microbial biofilm control.

P4-47 The Effect of Heat Treatment on the Antimicrobial Susceptibility Profiles of *Listeria monocytogenes* Scott A and *Listeria innocua*

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Introduction: Listeria monocytogenes in foods is currently a major concern among the food industry and consumers. However, it's reported that a number of factors (growth phase, temperature and pH) may influence the extent of heat shock response of microorganisms.

Purpose: In this study, the effect of heat stress on the growth and antibiotic profiles of *Listeria monocytogenes* Scott A, *L. innocua*, and a mutant strain of *Listeria monocytogenes* (Hly -) were determined.

Methods: To determine the effect of heat on the antibiotic resistance profiles of *Listeria*, overnight stationary phase cultures were subjected to heat at 55°C for 10 min and the antimicrobial susceptibility profiles were determined by the spiral gradient endpoint (SGE) method with 13 different antimicrobial agents at concentrations of 0.5 µg–512 µg/ml. Breakpoints for each antimicrobial agent were determined based on the recommendations of the Clinical and Laboratory Standards Institute.

Results: Approximately a 2 log reduction was observed for the heat stressed *Listeria* strains. Unstressed *Listeria monocytogenes* Scott A was resistant to 6 (46%) of the antibiotics, in contrast to heat stressed *L. monocytogenes* Scott A, which was resistant to only 2 (15%) of the antibiotics. With the 13 antibiotics tested, unstressed *L. innocua* and *L. monocytogenes* (Hly-) were resistant to 10 (77%) and 4 (31%), respectively, compared to heat stressed *L. monocytogenes* (Hly-), which exhibited resistance to 9 (69%) of the antibiotics.

Significance: The results of this study indicate that the use of heat as an intervention step in inactivating *Listeria* spp. may play an important role in the dissemination of multiple antibiotic resistant *Listeria* spp.

P4-48 Membrane Fatty Acid Changes of Cells from Ten *Listeria monocytogenes* Strains Exposed to Various Antimicrobials

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Introduction: Bacterial cell membranes include a lipid matrix within which proteins are interspersed. Fatty acids in the membrane can be profiled by gas-chromatography, using methanol extraction, which converts fatty acids to methyl esters. The fatty acid composition of cell membranes may be used to determine changes in fluidity due to antimicrobial exposure.

Purpose: This study examined the effects of antimicrobials (acids: 0.4% acetic, 0.3% citric, 0.4% lactic; salts: 0.1% sodium diacetate, 0.6% potassium lactate, 0.4% buffered-sodium-citrate; bacteriocins: 0.2% nisin, 0.2% pediocin; essential oils: 0.4% carvacrol, 0.4% eugenol, 0.4% thymol; fatty acids/esters: 0.02% monolaurin, 0.04% octanoic acid) on membrane fatty acid composition of *Listeria monocytogenes*.

Methods: *L. monocytogenes* (10 strains tested individually) were cultured (30°C/22 ± 2 h) in Tryptic Soy Broth with 0.6% yeast extract (TSBYE) and washed twice with phosphate-buffered-saline (PBS). Individual pellets were resuspended in TSBYE plus each antimicrobial and incubated (7°C/72 h). Cells were washed twice (PBS) and fatty acid methyl esters extracted by subjecting the pellet sequentially to a saponification solution (100°C/30 min), a methylation solution (80°C/10 min), an extraction solution (25°C/10 min), and an alkaline washing solution (25°C/5 min). Fatty acid methyl esters were analyzed on a Hewlett-Packard gas chromatograph (model 6890). The complete experiment of 130 combinations was replicated at a later date.

Results: Exposure to acids, salts and bacteriocins stimulated greater membrane fluidity as expressed by an increase in short-chain/branched fatty acid content. Cultures treated with essential oils and fatty acids/esters showed an increase in 20:0 fatty acid content, which also denotes reduced fluidity. Strains R2-500 and R2-501 generally demonstrated increased fluidity after antimicrobial exposure; in contrast R2-763, R2-764 and R2-765 did not.

Significance: In this investigation the effects of antimicrobials on membrane fatty acid composition varied depending on antimicrobial and *L. monocytogenes* strain. This information could be used to determine more efficient sequences or combinations of antimicrobials in hurdle systems.

P4-49 Phenotypic Characterization of gtcA Transposon Mutants of Serotype 4b *Listeria monocytogenes*

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Introduction: We have shown previously that gtcA transposon mutant strains of serotype 4b *Listeria monocytogenes*, which lack galactose on their teichoic acid, have reduced virulence in a murine model of gastrointestinal infection. In addition, gtcA transposon mutants are less able to invade and multiply within Caco-2 human intestinal epithelial cells and murine embryonic hepatocytes (TIB73). The impacts on virulence were observed with gtcA mutants of both an outbreak-associated strain (California outbreak) and a sporadic case serotype 4b strain.

Purpose: In this study, we investigated the impact of gtcA inactivation on selected attributes of relevance to food contamination by *Listeria*.

Methods: Swarming motility was assessed by measuring the diameter of bacterial growth on soft agar incubated at room temperature. Growth rates of gtcA transposon mutants of an outbreak-associated strain (F2365, California outbreak) and a sporadic case serotype 4b strain (4b1) at 10°C were compared with their parent strains in BHI broth and on sliced deli turkey meat. We also assessed resistance of bacteria to inactivation by synthetic gastric fluid (pH 4.5). Biofilm formation on plastic was assessed by staining of bacterial cells adherent to 96 well plastic plates.

Results: In soft agar motility assays, both mutants had reduced swarming, suggesting reduced motility or chemotaxis. Inactivation of gtcA did not affect growth of either mutant at 10°C in BHI broth. The gtcA mutant of the sporadic strain 4b1 grew more rapidly than the parental strain on sliced deli turkey meat incubated at 10°C, whereas growth of the mutant of the epidemic-associated strain was not affected. We also observed a difference in resistance to synthetic gastric fluid (pH 4.5) between the two gtcA mutants. Inactivation of gtcA resulted in enhanced susceptibility to synthetic gastric fluid only in the epidemic associated strain. Similarly, inactivation of gtcA was accompanied with decreased adherence to plastic (i.e. biofilm) only in the epidemic-associated strain.

Significance: These findings indicate that in serotype 4b *L. monocytogenes,* gtcA is important in several characteristics of relevance to contamination of foods, and that the impact of the gene on such attributes is strain-specific.

P4-50 The Impact of Cold Shock Family Proteins on Growth of *Listeria monocytogenes* at Low Temperatures and in Presence of Organic Acids

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Introduction: The ability to survive and proliferate under stress is an important attribute of the foodborne pathogen *Listeria monocytogenes*. The *csp* gene family of bacteria encodes small highly conserved nucleic acid-binding proteins, the cold shock proteins (CSPs), presumed to have roles in normal growth and stress adaptation responses. *L. monocytogenes* has three *csp* family genes (*cspA*, *cspB* and *cspD*) of presently unknown function.

Purpose: The purpose of this study was to evaluate the role of *L. monocytogenes cspA*, *cspB* and *cspD* s during growth under normal and stress conditions.

Methods: The growth of six single and double *csp* deletion mutant strains (Δ*cspA*, Δ*cspB*, Δ*cspD*, Δ*cspAB*, Δ*cspAD*, Δ*cspBD*) and the parental wild type *L. monocytogenes* EGD-e strain was evaluated at optimal and refrigeration temperatures (37°C, 10°C and 4°C), as well as in the presence of organic acids (pH 5.5–6.0).

Results: All *csp* deletion mutants and the parental wild type strain had similar growth phenotypes at 37°C. However, the D*cspA*, D*cspAD*, and D*cspAB* mutant strains failed to grow at 10 and 4°C, and the growth of D*cspD* and D*cspBD* strains was significantly impaired at 4°C compared to the wild type strain, whereas the D*cspB* strain continued to grow similarly to the parental wild type strain even under such cold stress conditions.

Poster

In the presence of organic acids (lactic, acetic and citric acids), the growth phenotypes of Δ*cspA*, Δ*cspD*, Δ*cspAB*, Δ*cspAD*, and Δ*cspDB* mutants were also slightly impaired compared to the wild type and the D*cspB* strains.

Significance: *L. monocytogenes* *cspA* and *cspD* encode functions that are required for optimal cold and acid stress adaptation responses in this organism. This might have some important implications for *L. monocytogenes* growth on refrigerated food products and in the presence of organic acid-based preservatives.

P4-51 DSC The Effect of Acid Stress and Heat Shock on the Minimum Ultraviolet Light Dose Required to Inactivate *Listeria monocytogenes* in Water and 9% NaCl

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Introduction: Bacteria exposed to sublethal stress may become more resistant to later applications of the same stress or other stresses.

Purpose: The purpose of this research was to determine if application of common food processing stresses (acid and heat) affects the dose of ultraviolet light required to significantly reduce the amount of *Listeria monocytogenes* in water and 9% NaCl.

Methods: *L. monocytogenes* strains were acid stressed (35°C for 3 h in TSBYE acidified to pH 5.0) and heat shocked (BHI at 48°C for 1 h) and suspended in water and 9% NaCl solution; each containing 10^{-4} M uridine. Fourteen ml of suspension was placed into a sterile quartz cell and exposed to ultraviolet light (UV) for 0, 5, 10, 15, 20, 25, or 30 min in a photoreactor fitted with a 254 nm pass-through filter. The sample was held at 8°C and continuously stirred during irradiation. Microbiological analysis was performed by serially diluting samples in 0.1% peptone, and surface plating onto MOX and TSAYE, or by enrichment in BHI followed by incubation at 37°C for 24 h. The UV dose was calculated by uridine actinometry.

Results: Acid-stressed *L. monocytogenes* in water decreased by 5.98 and 5.81 log CFU/ml as determined on TSAYE and MOX, respectively, after exposure to 43.89 mJ/cm² (30 min). Acid-stressed *L. monocytogenes* in 9% NaCl decreased by 5.95 and 4.45 log CFU/ml on TSAYE and MOX, respectively, after UV exposure to greater than 32.57 mJ/cm² (30 min). Heat-shocked *L. monocytogenes* in water decreased to below the detection limit (1 log CFU/ml) at UV doses greater than 13.14 mJ/cm² (20 min) but was detected via enrichment after exposure of up to 24.78 mJ/cm² (30 min). Heat-shocked *L. monocytogenes* in 9% NaCl decreased to below the detection limit after exposure to a dose greater than 20.41 mJ/cm² (30 min).

Significance: Acid-stressed *L. monocytogenes* were more resistant to UV than heat-shocked cells, indicating that prior stress may affect the efficacy of UV treatments for reduction of this pathogen.

P4-52 Invasiveness of Non-Starved and Up-to-24-Month Starvation-Stressed Cells of *Listeria monocytogenes* Scott A Serotype 4b in the Human Caco-2 Cell Model

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Introduction: *Listeria monocytogenes* is known to persist in a wide variety of potential reservoirs and sources in food processing plants and it is important to understand the virulence potential of this organism when stress conditions are induced.

Purpose: Our aim is to examine the difference in virulence of non-starved and long-term starvation-stressed cells of *L. monocytogenes* by the human Caco-2 cell model.

Methods: For the induction of starvation stress, *L. monocytogenes* serotype 4b cells were grown to 10^9 CFU/ml in TSBYE, washed twice by centrifugation, resuspended in 0.85% NaCl, and held at room temperature for up to 24 months. Invasiveness or internalization in Caco-2 cells by non-starved and long-term starvation-stressed cells of *Listeria monocytogenes* serotype 4b were determined by challenging 10^3 to 10^7 cells/ml of *L. monocytogenes* onto 10^5 to 10^6 cells/ml of Caco-2.

Results: The CFU numbers of *L. monocytogenes* gradually decreased to 10^2–10^3 CFU/ml during 24 months of starvation stress from the original 10^9 CFU/ml concentration. The numbers of *L. monocytogenes* internalized in Caco-2 increased with increases in infection time (from 1 to 4 h) and in infection dose (MOE of *L. monocytogenes*: Caco-2 from 1:10 to 100:1) both for non-starved cells and starvation-stressed cells. After 4 h of infection at the different MOE tested, starvation-stressed cells (1 to 24 months) of *L. monocytogenes* were internalized in Caco-2, but there were 1-4 log fewer counts as compared to counts for non-starved cells. Within 24 h after internalization, starvation-stressed cells (1 to 24 months) of *L. monocytogenes* increased to about 3–6 $\log_{10}$ CFU/well in Caco-2 versus increases to 7 $\log_{10}$ CFU/well for the non-starved cells.

Significance: These studies indicate that in spite of long-term starvation stress for up to 24 months in physiological saline, the surviving population of *L. monocytogenes* serotype 4b cells still remained infective to human Caco-2 cells.

P4-53 DSC Comparison of Antimicrobial Resistance Determinants among *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* Isolated from Swine

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Introduction: The importance of *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus* as carriers of antimicrobial resistance is well known, but little work has been done to examine the relationship between this phenotypic characteristic and genotypic attributes among strains isolated in similar settings and time points.

Purpose: The purpose of this project is to determine the role of peri-harvest factors on selection and dissemination of antimicrobial resistant foodborne and commensal organisms.

Methods: Isolates were collected from processing plants in North Carolina and Iowa. Samples originated from the head meat, carcass, lairage swabs, and lymph nodes. Phenotypic characterization of antimicrobial resistance patterns was completed on 1,284 samples. Genotypic characterization by PCR and sequencing of resistance genes and class-1 integrons was performed on 128 isolates selected based on multidrug resistance pattern and association with other samples.

Results: Phenotypically, all four pathogens were highly resistant to tetracycline, with the lowest prevalence, being in *Salmonella* at 69.4%. Genotypically, the most common genes found are tetA(B) (26.6%), aadA2 (19.5%), and cmlA (18.0%). Within the samples that tested positive for tetA(B), aadE, and cmlA there were multiple groups of organisms that were from the same animal. Class-1 integrons were found in 43.75% of the samples with the majority found in *Salmonella* (22.7%) and *Enterococcus* (19.5%). Large (4kb) integrons were found in *Salmonella* serovar Havana and *Enterococcus* samples, in contrast to previous reports.

Significance: The overlapping of resistance genes found within the four organisms may be the result of horizontal resistance gene transfer within the host. Large integrons among MDR *Salmonella* and enterococci is also of high significance since integrons facilitate the carriage and dissemination of multi-drug resistance.

P4-54 Phenotypic and Genotypic Characterization of Multi-Drug-Resistant *Salmonella* Serotype Heidelberg Isolated from Humans and Animals

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Introduction: Salmonella enterica is one of the common bacterial pathogens causing foodborne illnesses worldwide. In the United States alone, *Salmonella* serotype Heidelberg has been recognized as the fourth most common *Salmonella* serotype that causes foodborne diseases, with an estimate of almost 2,000 illnesses. This serotype is also commonly identified from swine.

Purpose: The purposes of the study were to determine and compare the frequency of antimicrobial resistance of *Salmonella* Heidelberg among various host species and to identify the predominant phenotypes and genotypic diversity of *Salmonella* Heidelberg using antimicrobial resistance patterns and genotypes.

Methods: Salmonella Heidelberg isolated from humans (47), swine (45), and turkeys (3) were tested for susceptibility against 12 antimicrobial agents, using the Kirby-Bauer method. Genotyping was performed by PFGE. Antimicrobial resistant genes and class I integron were detected by PCR. Sequencing was performed after the amplicon of the class I integron from PCR product was purified following the manufacturer's protocol.

Results: No pan-susceptible strains were detected among swine isolates, and the majority (75%) of swine isolates showed multi-resistant against kanamycin, streptomycin and tetracycline (KmStTe). About 80% of *Salmonella* Heidelberg isolated from humans were pan-susceptible. One isolate showed resistance to 10 of the 12 antimicrobials tested. PFGE showed diversity among the majority of the isolates regardless of host origin. Resistance genes detected include aphA1-Iab, strA, blaTEM, aadA2, tetA(B), and class I integron. DNA sequencing revealed that both human and swine isolates carried aadA2 on a class I integron. Two *Salmonella* strains from swine were also found to carry sat and dfrA12 resistant gene cassettes within class I integron.

Significance: The results obtained by this study suggest that MDR Heidelberg is common and is a public health concern. MDR strains were isolated more commonly from swine. *Salmonella* Heidelberg appeared to have aadA, sat, and dfrA integrated in the class I integron.

P4-55 Influence of Autoinducer-2 (A1-2) on the Growth and Virulence of *Salmonella enterica* Serovar Typhimurium and Modulation of These Effects from Poultry Meat-Derived Fatty Acids Having AI-2 Inhibitory Properties

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Introduction: Salmonella Typhimurium is an enteric pathogen which can act as an agent of foodborne illness and is primarily associated with animal products, particularly poultry. A signaling compound produced primarily by Gram negative bacteria for cell-to-cell communication is autoinducer 2 (AI-2). Studies have indicated that AI-2 has an impact on bacterial virulence.

Purpose: We have shown that poultry meat-derived fatty acids can interfere with AI-2 function. The focus of this study was to determine the influence of AI-2 on the growth and virulence of *Salmonella enterica* serovar Typhimurium, and whether fatty acids could modulate the effects of AI-2.

Methods: A *Salmonella enterica* serovar Typhimurium luxS mutant was grown in M-9 minimal medium supplemented with glucose and long-chain fatty acids (FA) of varying concentrations. In addition, treatments were supplemented with in vitro synthesized AI-2 or PBS. After a 12 h incubation during which OD_{600} values, were recorded, comparative analysis was done, calculating the growth constants for each treatment. Bacterial invasiveness, using a murine macrophage cell line, RAW 264.7, was also investigated.

Results: No significant difference was seen in the combined FA + AI-2 treatments compared to the AI-2 treatment. The majority of FA treatments had no impact on the growth of *Salmonella.* However, there was a significant increase in the growth rate with the AI-2 treatments when compared to the PBS control ($P = 0.01$). AI-2 decreased cell invasiveness ($P = 0.02$), while the addition of combined FA restored invasiveness to normal levels.

Significance: Though fatty acids have AI-2 inhibitory effects, the growth and virulence of *Salmonella* is not modulated by these fatty acids. This study establishes that AI-2 can have have varying effects on *Salmonella,* namely enhancing the growth of *Salmonella* (in minimal medium) to decreasing the infectivity of macrophages.

P4-56 DSC Induction of Cross-Protected and Viable but Nonculturable *Salmonella enterica* Serotype Typhimurium under Various Stress Conditions

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Introduction: Foodborne pathogens can be adapted to acid and cold temperature, which can cause increased resistance to other food preservatives and eventually lead to serious illness. The potential risk of cross-protected and viable but nonculturable (VBNC) pathogens is of great concern to food safety.

Purpose: The objective of this study was to evaluate the VBNC phenomenon in cross-protected *Salmonella enterica* under acid and cold stresses.

Methods: For acid and cold challenge experiments, approximately 10^7 CFU/ml of *S. enterica* were adapted at 37°C for 0, 2, and 7 h in Trypticase Soy Broth (TSB) adjusted to pH 5.0 with acetic acid. The acid-adapted cells were incubated in TSB (PPH 4.0) at 4 and 20°C until cultures became negative. Recovery of injured cells was determined by plating on TSA and XLD agar. Double staining with fluorescein isothiocyanate and propidium iodide was performed to differentiate viable and dead cells by flow cytometry. Proteins expressed in non- and acid-adapted cells were analyzed by SDS-PAGE.

Results: The acid-adapted *S. enterica* cells were more resistant to lethal acid and cold stresses than the non-acid-adapted cells. The numbers of non-acid-adapted cells were reduced by 4.32 and 7.55 log CFU/ml after 6 days of incubation at 4°C and 20°C, respectively, whereas those of acid-adapted cells were reduced by 1.17 and 6.25 log CFU/ml. The numbers of injured cells were significantly increased ($P < 0.05$) in the non-acid-adapted cells throughout the incubation time. The non-acid-adapted cells lost culturability on day 8 after lethal acid challenge. When compared with the non-acid-adapted cells, the acid-adapted cells showed noticeable changes in outer membrane protein profiles.

Significance: These results provide practical information on the induction of cross-protected and VBNC pathogens under various stresses, which might be needed to design new food preservation strategies and reduce potential health risk.

P4-57 Simple, Rapid and Reliable Detection of Enterophaemorrhagic *Escherichia coli* O26 Using Immunochromatography

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Introduction: Food-manufacturing establishment needs simple, rapid and reliable tools monitoring foodborne pathogens. Various pathogen screening kits including our NH Immunochromatography *E. coli* O157, *Listeria* and *Salmonella* are available, but no rapid and reliable screening kit for *E. coli* O26 has been commercially provided.

Purpose: The present study was carried out to construct an immunochromatography detecting *E. coli* O26 and to evaluate its sensitivity and specificity.

Methods: The immunochromatography detecting *E. coli* O26 was prepared using anti-O26 polyclonal antibody. One hundred-µl portions of serial 10-fold dilutions of overnight cultures of *E. coli* O26, other O-serotype *E. coli* and *Enterobacteriaceae* were applied to the immunochromatography. The immunochromatography was also provided to detection of *E. coli* O26 from experimentally inoculated ground beef.

Results: The present immunochromatography was able to specifically detect *E. coli* O26 of 1×10^4 to 1×10^5 CFU/ml and show no cross reaction with bacteria other than *E. coli* O26 tested. Moreover, the present immunochromatography detected *E. coli* O26 inoculated at a level of 1 to 10 CFU/25 g of ground beef in only 18 h of enrichment.

Significance: These results show that the present immunochromatography is a simple, rapid and reliable tool screening for *E. coli* O26.

P4-58 Evaluation of Eukaryotic Cell Invasion on a Library of Genetically Diverse *Campylobacter* spp. Isolates

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Introduction: Campylobacter spp. are the largest cause of sporadic bacterial gastrointestinal infection in the industrialized world. Epithelial cell invasion is thought to be necessary to bring about infection in humans. Invasion studies have shown that different *Campylobacter jejuni* isolates may differ in their ability to invade human cells. Non-invasive strains were isolated from patients with noninflammatory disease, while invasive strains have been isolated from patients with inflammatory diarrhea.

Purpose: The purpose of this study was to further evaluate *Campylobacter* isolates obtained from various sources on their ability to adhere and invade Caco-2 cells (human colon cells).

Methods: In an effort to further investigate genetic variability [based on flaA short variable region (SVR) DNA sequence] and host specificity (humans, broilers, and breeders), 52 *Campylobacter* isolates originally recovered from Iceland (with well defined spatial and temporal backgrounds) were employed in invasion assays with Caco-2 cells, to determine whether the flaA SVR sequence analysis of *Campylobacter* spp. is indicative of potential virulence propertles such as cell adherence and invasion.

Results: The range of adhesion was from 0.00008% to 3.4%. The range of invasion was 0.000003% to 1.2%. The control, *C. jejuni* 81-176, had a percent adhesion rate of 0.083% and invasion rate of 0.75%. No correlation was found between the flaA SVR and cell adhesion and invasion, thus suggesting that invasiveness cannot be predicted on flaA SVR alone.

Significance: The flaA SVR is not a good predictor of the adhesion and invasiveness properties of *Campylobacter*. Additional DNA sequence investigations such as these are needed to further delineate mechanisms involved in *Campylobacter* spp. infection in humans.

P4-59 DSC Thermal Inactivation of *Campylobacter jejuni* in Broth

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Introduction: New Zealand has a high reported incidence rate of campylobacteriosis and a number of studies have indicated that poultry meat consumption is a major risk factor. However, *C. jejuni* has been demonstrated to be heat sensitive, and proper cooking will eliminate all viable cells. It has been suggested that the high rate of illness in New Zealand may be partly due to the emergence of heat resistant strains. In addition, the remarkably high D values reported by Bergsma (2007) need further investigation.

Purpose: The objective of this study was to determine the thermal inactivation kinetic parameters (D and z) for selected strains of *C. jejuni* isolated in New Zealand from humans and poultry.

Methods: Campylobacter MLST strains commonly isolated from local poultry products and from human cases were heated in BHI broth to a predetermined temperature, using a submerged coil heating apparatus.

The inoculum and the number of surviving organisms in the sample suspensions after heat treatment were determined by dilution in BHI broth followed by plating on mCCDA, using an automated spiral plater. Survival curves were plotted and D-values calculated and compared by use of ANOVA.

Results: D-values were obtained at temperatures of 51.5 to 60°C for seven relevant New Zealand *C. jejuni* strains. The range of D values and the z values for the isolates were 300 s – 2 s and 4.0 – 4.7°C respectively, for this temperature range. Variations in the observed D values appeared to be source-independent and due to natural biological variation. These D & Z values are in broad agreement with published international data and do not indicate that New Zealand *Campylobacter* strains are unusually heat resistant

Significance: The results provide robust data for use in quantitative risk assessment but do not support the hypothesis that the most prevalent New Zealand strains are unusually heat resistant.

P4-60 Antimicrobial Resistance, Virulence and Genotypic Profiling of *Campylobacter jejuni* and *C. coli* Isolated from Humans and Retail Meat

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Introduction: Campylobacter is the leading cause of bacterial gastroenteritis, with an estimated 2.5 million cases annually in the US. *C. jejuni* in humans is considered to be the most important species causing 95% of foodborne infections. It is important to characterize *C. jejuni* and *C. coli* at the phenotypic and genotypic levels to aid in outbreak investigations.

Purpose: The aim of this study was to characterize 361 *Campylobacter* isolates, including *C. jejuni* (n = 263) and *C. coli* (n = 98) isolates from humans and retail meat to determine if they were related in their virulence, antimicrobial resistance and genotypic profiles.

Methods: Campylobacter isolates were tested by PCR for the presence of 9 virulence genes (flaA, cadF, rakR, cdtA, cdtB, cdtC, dnaJ, pldA, virB11). Antimicrobial susceptibility profile to four antimicrobials was generated, using the agar dilution method, and pulsed field gel electrophoresis (PFGE) with two enzymes was used for genotyping.

Results: Antimicrobial resistance to ciprofloxacin and erythromycin in *C. coli* was observed in 29.4% and 37% isolates from retail meat and in 15% and 55 % isolates from humans, respectively. Fluoroquinolone resistance in *C. jejuni* was observed in 15% and 18% of the isolates from humans and chicken, respectively. Virulence determinants were more prevalent in isolates from retail meat and 95% of isolates were positive to 3 or more genes. Clonality was observed between *C. jejuni* isolates from humans and chicken and better discrimination was observed using two enzymes.

Significance: The results indicate that *C. coli* isolates from humans and retail meat are more virulent than *C. jejuni*. High frequency of antimicrobial resistance observed in *Campylobacter* isolates against fluoroquinoloes and aminoglycoside is of concern from the food safety perspective. Genotyping showed evidence of *C. jejuni* transmission between chicken and humans, which can have important public health implications.

P4-61 Isolation and Genotyping of *Enterobacter sakazakii* from Korean Traditional Powdered Foods (Sunsik) and Related Raw Materials

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Introduction: Enterobacter sakazakii, known as yellow-pigmented *Enterobacter cloacea*, causes life-threatening meningitis, septicemia, and necrotizing enterocolitis in infants. Although the mode of transmission of *E. sakazakii* has not been clearly identified, powdered infant formula may be the vehicle of the organism. The ingredients of sunsik, a Korean traditional powdered health food, are grains, beans, vegetables and seaweeds. Some infants have been fed sunsik as a weaning diet in Korea.

Purpose: The purpose of this study was to determine the presence of *E. sakazakii* in sunsik and related raw materials and analyze the characteristics and genotyping of the isolated strains.

Methods: Isolation of *E. sakazakii* from sunsik and related raw materials was carried out using Druggan-Forsythe-Iversen medium and identified using the Vitek GNI card. Amplification of the 16S rRNA gene from the organism isolates was conducted with Esakf and Esakr primer pairs. The antibiotic susceptibility and genotyping of the isolates were determined by the Vitek system and randomly amplified polymorphic DNA (RAPD)-PCR using UBC 245 primer.

Results: E. sakazakii was isolated from 9 out of 15 sunsik samples and 26 out of 72 related raw materials. Contamination of root vegetables such as potato, carrot, yam and onion was over 50% higher than that of other related raw materials. All isolates showed a 929 bp band amplified from 16S rRNA and resistant to ampicillin, amoxicillin/clavulanic acid and cefazolin. The banding patterns conducted by RAPD-PCR were diverse.

Significance: This study revealed that sunsik is not free of *E. sakazakii* and needs to be monitored continually. The practice of sunsik feeding to infants should be handled with care.

P4-62 DSC Invasion of *Enterobacter sakazakii* Strains MNW2 and SK81 in Neonatal Mice

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Introduction: Enterobacter sakazakii (*E. sakazakii*) has been associated with outbreaks of infection in neonatal intensive care units (NICUs) among premature or very-low-birth-weight infants fed contaminated powdered infant formula. *E. sakazakii* infection can result in severe illnesses such as septicemia, meningitis or hydrocephalus or even in death.

Purpose: Our objectives were to (1) compare the susceptibilities of three mouse strains to *E. sakazakii* by observing mortality and infectivity, (2) compare virulence of *E. sakazakii* strains MNW2 and SK81, and (3) compare the susceptibilities of male and female neonates challenged with *E. sakazakii* strain SK81.

Methods: Timed-pregnant mice of the CD-1, BALB/C and C57BL/6 strains were obtained, acclimatized, and allowed to give birth naturally. At postnatal day (PND) 3 or 4, the pups were orally gavaged with a single dose of vehicle or 10^2–10^{12} colony-forming units (CFU) *E. sakazakii* per ml reconstituted powdered infant formula. Pups surviving to PND 10 or 11 were sacrificed and brains, livers, and ceca excised and analyzed for the presence of *E. sakazakii*.

Results: The CD-1 mouse strain was the most susceptible of the three, demonstrating a dose-dependent response in mortality and the lowest infectious dose of 10^2 CFU/ml for all tissues. Comparing the invasion of liver and brain tissues after treatment of pups with 10^5 CFU MNW2 and SK81 indicates that MNW2 is more invasive in both liver (43% versus 8%) and brain (71% versus 35%) than SK81. There appeared to be no significant difference in the susceptibilities of male and female CD-1 neonates administered *E. sakazakii* strain SK81.

Significance: Understanding and developing animal models for *E. sakazakii* infection will allow development of therapies to treat *E. sakazakii* infections. Comparisons of different strains of *E. sakazakii* will help determine which are more virulent and likely to cause morbidity and mortality in premature infants.

P4-63 Isolation and Characterization of Enterotoxigenic *Staphylococcus* Strains from Cheese in Bogota, Colombia

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Introduction: In Colombia the most important pathogens associated with foodborne illness are *Staphylococcus aureus* and *Salmonella* sp. They rank first and second for prevalence of foodborne illness in annual epidemiologic reports.

Purpose: Because *Staphylococcus* is one of the principal foodborne pathogens in our country, the aim of this study was to identify and characterize *Staphylococcus aureus* enterotoxigenic strains from cheese obtained from different retail outlets, supermarket stores and street food vendors in Bogota, Colombia.

Methods: Staphylococcus spp. were isolated from cheeses from retail outlets, supermarket stores and street food vendors. PCR to detect the SEA gene was performed, using the primers previously reported. A total of 50 cheeses were analyzed corresponding to 13 sold by street food vendors and retail outlets and 37 from supermarket stores.

Results: Staphylococcus aureus was isolated from the whole cheese samples from retail outlets and street food vendors. There were no *Staphylococcus* spp. from supermarket stores samples (n = 37). All of the isolates were positive for the SEA.

Significance: There are differences in cheeses depending on their sales channels. This study revealed a very high frequency of *Staphylococcus aureus* coagulase-positive and enterotoxin A gene strains from cheeses sold by retail outlets and street food vendors.

P4-64 Enterotoxigenicity and Other Metabolic Characteristics of *Staphylococcus* Species

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Introduction: Staphylococcal food poisoning is a commonly reported illness caused by the ingestion of preformed enterotoxin in foods produced by some strains of staphylococci.

Purpose: While this type of illness is almost exclusively associated with *Staphylococcus aureus*, other species of staphylococci can be enterotoxigenic, thus having the potential of being the etiological agent of foodborne illness.

Methods: Seventeen randomly selected strains of staphylococci were purchased from the American Type Culture Collection (ATCC). Cultures were grown in brain heart infusion broth, pH 5.5, for 24 h at 35 degrees centigrade on a shaker. Culture fluids were collected by centrifugation before assay for enterotoxin presence.

Results: All cultures which showed growth were assayed for staphylococcal enterotoxin (SE) production for serotypes A-E, using ELISA-based methods. Toxin negative culture extracts were concentrated 10X; then the concentrates were reassayed for toxin presence. Of the 13 ATCC cultures producing growth, eight were enterotoxigenic for one or more serotypes SEA/B/C/D/E and showed diverse patterns of other metabolic events such as coagulase, nuclease, hemolysin and carbohydrate fermentation.

Significance: These findings indicate that staphylococcal species other than the typical *S. aureus* should be considered when investigating foods for potential foodborne pathogens and when examining foods for preformed enterotoxin containing large numbers of non-aureus staphylococci.

P4-65 The Distribution of Newly Described Enterotoxin-Like Genes in *Staphylococcus aureus* from Ready-to-Eat Food in Korea

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Introduction: Staphylococcus aureus is an important pathogen isolated from cases of community- and hospital-acquired infection. *S. aureus* produces a variety of staphylococcal enterotoxins (SEs) that are causative agents of staphylococcal food poisoning (SFP). Recent studies of the *S. aureus* genome resulted in the discovery of a number of new enterotoxin homologues, designated as staphylococcal enterotoxin-like toxins (SEIs).

Purpose: We investigated the distribution of SEI genes among *S. aureus* isolated from Ready-to-Eat food in Korea.

Methods: A total of 154 *S. aureus* were examined for the presence of nine SEI genes (seik to seir, and selu) by multiplex polymerase chain reaction (PCR) using primers designed on the basis of dual priming oligonucleotide (DPO) technology.

Results: All nine SEI genes were detected among *S. aureus* isolates. Seventy-nine (51%) of all isolates possessed at least one of the detected SEI genes. The prevalent SEI genotype of isolates was sek+seq (21%), sem+sen+seo (10%), and sem+sen+seu (8%). The frequently detected genes were selq (23%), selk (22%), selm (22%), and seln (22%). As a result of comparison with SE genotype of tested isolates, 95% of isolates having SEI genes were shown to carry SE genes, although four isolates having selp did not possess any of SE genes. Both selk and selq were detected in almost all (32 of 33 Isolates, 97%) of isolates having sea+seh genotype that is the major genotype of *S. aureus* from food. Also, all isolates (34 isolates) having seg and sei contained selm and seln. However, 89% of Isolates (17

of 19 isolates) having sea solely did not have SEI genes at all.

Significance: Our results demonstrated the high prevalence of the newly described SEI genes and frequency of coexistence of SE and SEI genes in *S. aureus* from food in Korea.

P4-66 Morphological Analysis of Heat-Sensitive and Heat-Resistant Spores of *Clostridium sporogenes* via Transmission Electron Microscopy

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Introduction: Clostridium sporogenes PA 3679, a non-pathogenic, putrefactive and spore-forming anaerobe, is an important microorganism used as a surrogate for modeling thermal inactivation of *C. botulinum*. Thus, refined understanding of the heat resistance of *C. sporogenes* spores and its mechanisms are helpful in establishing sterilization processes applicable to inactivate *C. botulinum*.

Purpose: This study was performed to investigate the primary structural determinants affecting heat resistance of *C. sporogenes* spores.

Methods: The shapes of the structural components of heat-sensitive and heat-resistant spores before and after heat treatment were observed and compared via transmission electron microscopy.

Results: The mean thickness (± SD) of coat layers and cortex regions of heat-sensitive spores were 82.9 ± 14.5 and 86.0 ± 22.7 nm, while those of heat-resistant spores were 106.9 ± 45.7 and 111.7 ± 32.1 nm, respectively. The thickness of coat ($P = 0.031$) and cortex ($P = 0.006$) showed statistically significant differences, suggesting that heat-resistant spores have a thicker coat and cortex than heat-sensitive spores. The mean sizes (± SD) of cores were 467.0 ± 88.7 nm for heat-sensitive spores and 460.2 ± 98.5 nm for heat-resistant spores, respectively, which showed no statistically significant differences. The ratios (± SD) of the core size to the sporoplast size were 0.84 ± 0.05 for heat-sensitive spores and 0.80 ± 0.07 for heat-resistance spores, respectively, showing statistically significant differences ($P = 0.030$), which indicated that the ratio is negatively related to heat resistance. Accordingly, the structural components of heat-sensitive spores were severely damaged by heat treatment, whereas those of heat-resistant spores were unlysed under the same conditions.

Significance: These data suggest that the thickness of coat layer and cortex region are significantly correlated with heat resistance of *C. sporogenes* spores and that cortex region plays a major role in protecting the spore from heat damage.

P4-67 Effect of the Combination of pH, Water Activity and Temperature on the Germination of *Bacillus anthracis* Spores

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Introduction: There is little information about the behavior of *Bacillus anthracis* in foods.

Purpose: In this study we examined the combined effects of pH, water activity and temperature commonly associated with foods on the germination of *B. anthracis* spores.

Methods: Brain heart infusion broths of 15 combinations of pH (4.6–8.0) and water activity (0.900–0.984) were used in the study. There were five tubes for each combination and each temperature. All tubes were inoculated with *B. anthracis* spores (Sterne, 3 log/ml) and incubated at 10, 20, 30, 37 and 42°C. Turbidity, an indicator of growth, was used as the indicator of spore germination and growth.

Results: After 10 weeks, there was no indication of spore germination in any a_w/pH combinations incubated at 10°C. Broths with $a_w < 0.919$ did not support the germination of *B. anthracis* spores at any temperatures or pHs tested. Broths of the following combinations (a_w/pH) showed positive growth of *B. anthracis* that indicated the germination of spores during the incubation: 0.984/8.0, 0.959/7.0, and 0.94/8.0 at 20°C; 0.984/8.0, 0.984/5.9, 0.984/4.6, 0.959/7.1, 0.959/5.3, 0.94/8.0, and 0.94/6.1 at 30°C; 0.984/8.0, 0.984/5.9, 0.959/7.1, 0.94/8.0, 0.94/6.1 and 0.94/4.6 at and 37°C; and 0.984/8.0 and 0.959/7.1 at 42°C.

Significance: Our results showed that lower temperature (10°C) or lower a_w (< 0.919) prevented the germination of *B. anthracis* (Sterne). Growth was observed in this study from spores in broth of 0.984/4.6 at 30°C and broth of 0.94/4.6 at 37°C; similar combinations did not support the growth of a vegetative cell inoculum in a previous study. This suggested that vegetative cells germinated from spores may be able to adapt to a less favorable environment better than cells grown from a more favorable environment. Knowledge of the factors affecting the germination and subsequent growth of *B. anthracis* spores will provide valuable information to evaluate the potential hazard if a food were contaminated with this pathogen.

P4-68 Development of Intervention Strategies for Pathogens in Protected Environments on Food-Contact Surfaces

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Introduction: With an estimated 76 million foodborne illnesses occurring each year due to pathogens, research has focused on control strategies to identify sources of contamination and to establish preventative measures. Identified sources include the surface of foods and the topographical features of food processing equipment (e.g., crevices, joints, or seals) that can trap pathogens and protect them from antimicrobial agents and convective shear forces associated with cleaning procedures.

Purpose: The purpose was to characterize protected environments on food-contact surfaces and to study their ability to harbor pathogens and protect them from intervention techniques.

Methods: COMSOL Multiphysics 3.2 was used to examine the effects of disinfectants diffusion in biofilms in grooves with ranging widths and depths and to model the flow patterns of different fluid cell geometries. Results from the models were used to machine fluid cells consisting of stainless steel surfaces with and without machined grooves. Bioluminescent *Escherichia coli* O157:H7 lux from pfSP102 biofilms were then grown via a flow through system in those fluid cells. Biofilm development was monitored by bioluminescence via photon multiplier tubes and low light level cameras. Once stable, biofilms were treated with a killing agent (e.g., bleach, chlorine dioxide), and efficacy is estimated by off-line growth assays such as plate counts.

Results: Groove width had minimal effect on disinfectant diffusion and potentially on microorganism survival when width ranged from 100 µm to 800 µm, whereas slit depth (50 µm – 300 µm) affected the mass transfer of diffusion. Using the optimized geometry for the fluid cell, we have shown that stainless steel surfaces with grooves yielded increased survival, and we are developing methodology to better quantify survival under these conditions.

Significance: Our findings will provide new methodologies and models to optimize treatment regiments to effectively eliminate pathogens in regions where they are most difficult to kill.

P4-69 Isolation of Bacteriophages Infecting Gram-Positive Foodborne Pathogens

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Introduction: There are now several publications reporting the inactivation by bacteriophages (phages) of foodborne pathogens in foods. Most of this work has focused on Gram-negative pathogens, with the notable exception of *Listeria monocytogenes*.

Purpose: The purpose was to isolate phages infecting *Bacillus cereus* and *L. monocytogenes* and evaluate their use for biocontrol of these foodborne pathogens.

Methods: Phages were isolated using the agar overlay technique and plaque purification. Structure was determined by electron microscopy, and genome size estimates from restriction digests of the DNA. Host ranges were ascertained using the spot test and single-step growth curves used to obtain growth cycle data.

Results: Two phages infecting *B. cereus* were isolated from a soil sample. Their appearance in electron micrographs was consistent with their being myoviruses, and they produced complete lysis of *B. cereus* in broth culture and mashed potato. They had a similar host range, infecting the same isolates of *B. cereus*, *B. thuringiensis* and *B. mycoides*. The genome size for both was approximately 134 kbp as determined by HinfI, SSPI, DraI and TaqI restriction enzyme patterns. At 37°C there was a 84–93 min latent period and a burst size of 300–332. Phages infecting *L. monocytogenes* were isolated from ruminant feces and were morphologically similar to typing phage A511, although slight differences in the length of the contracted tail sheath were noted. These phages also completely lysed a liquid culture of the pathogen when incubated at 30°C. They lysed all eight isolates tested, while phage A511 was unable to lyse one strain that is responsible for a significant proportion of listeriosis cases in New Zealand. Lysis of four other *Listeria* spp. was not uniform.

Significance: These isolates may provide new options for foodborne pathogen biocontrol. The *L. monocytogenes* phages infect a strain prominent among New Zealand clinical isolates.

P4-70 Potential Use of Bacteriophages to Control Pathogens in Foods Stored under Refrigeration

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Introduction: Most foodborne pathogens do not grow at refrigeration temperatures. Since phage replication occurs in a growing host, phage control of pathogens in refrigerated foods faces some challenges. However, "lysis from without" (LWO) could be used as a control if phages are applied in high numbers. Alternatively, since food which causes disease inevitably warms to 37°C, adding phages to hosts prior to refrigerated storage, thus priming them for lysis on warming, could be a control option.

Purpose: Two application strategies for control of pathogens during refrigeration were examined with two phages, one (P7) infecting *Salmonella* Typhimurium and the other coliphage T4.

Methods: Experiments were conducted in which cultures containing phage and host were maintained at 5°C prior to shift to 24°C and 37°C. LWO was measured from the reduction of optical density of a host suspension. Ghost phages were produced by use of UV inactivation, and pathogen numbers counted by spread plating. Host cells were enumerated by plate counts.

Results: Previous data showed that *Salmonella* was inactivated on meat by P7 at 5°C, but in vitro spectrophotometric data were inconsistent with LWO. Ghost T4 phages applied at high multiplicity of infection (MOI) produced a reduction in *Escherichia coli* numbers at 37°C but ghost P7 phages did not kill their host. Viable phages at equivalent MOI resulted in significant inactivation. No changes in *E. coli* or T4 numbers occurred when incubation was at 5°C, but when cultures were shifted from 5°C to 24°C or 37°C, host lysis occurred only in the presence of phages.

Significance: We conclude that infection followed by deferred lysis is more efficient than LWO for reducing host numbers following refrigerated storage for these two phages. However, LWO was not optimized and we have no information on the degree to which the function of ghost phages was affected by UV exposure.

P4-71 DSC Attachment and Chemical Inactivation of Noroviruses to Fomites

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Introduction: In spite of the application of strict sanitary measures, the incidence of food poisoning is not decreasing. Among the pathogens known to cause foodborne illnesses, most are noroviruses. These enteric viruses are transmitted via the fecal-oral route, with water, food and environmental fomites being the main vehicles.

Purpose: The objectives of this study were to evaluate the effectiveness of different disinfectants to inactivate noroviruses attached to fomites and to evaluate the impact of pH and relative humidity on their attachment to fomites.

Methods: An enzymatic pretreatment was performed on both human and murine noroviruses before their detection by real-time RT-PCR, to avoid detection of viral RNA associated with inactivated viruses. An animal-cell-based assay was used to observe the number of plaque-forming units of virus dried on a stainless steel surface for the murine norovirus and was then compared to the molecular method.

Results: The attachment of murine norovirus to stainless steel surfaces seems to be more affected at basic pH than at acid or neutral pH under low relative humidity. For the chemical inactivation, our results have shown that the sodium hypochlorite disinfectant was the most effective, with a more than 3-log reduction after 10 min compared to less than 1-log for quaternary ammonium compounds and ethoxylated alcohols. Murine norovirus seemed to be more sensitive than human norovirus and the molecular method has a better sensibility than plaque assays.

Significance: These results will help improve strategies for decontaminating surfaces harboring norovirus-

es and thus reduce the transmission of illness caused by these pathogens in the food sector and domestic environments.

P4-72 DSC Effect of Sodium Hypochlorite on Murine Norovirus, a Surrogate for the Human Norovirus

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Introduction: Norovirus is an important cause of foodborne illness worldwide, therefore disinfection and sanitation strategies are important issues for the food industry. Sodium hypochlorite is a sanitizer used widely throughout the food industry, e.g. for surface sanitation and in commercial produce washing. Murine norovirus (MNV-1), a human norovirus surrogate, is culturable and can withstand prolonged exposure to low pH. Determining the sensitivity of MNV-1 to sodium hypochlorite will provide information on whether current treatments used in produce washing are adequate to inactivate viruses.

Purpose: To investigate the resistance to sodium hypochlorite of MNV-1 in buffered suspension, in inoculated produce wash water, and on inoculated produce.

Methods: MNV-1 stock (approximately 6-$\log_{10}$ PFU/ml), suspended in phosphate-buffered saline (PBS) or lettuce homogenate, was added, at 1:10 (v/v), to 25 or 100 ppm sodium hypochlorite prepared in deionized water. Treatment times of 15 s to 60 s were investigated prior to neutralization with 1.8% sodium thiosulfate. Treatments were performed in duplicate at 20°C and 10°C.

Results: MNV-1 suspended in PBS was inactivated by >5-$\log_{10}$ PFU/ml after 15 s exposure to 25 or 100 ppm sodium hypochlorite at each temperature tested. When MNV-1 was suspended in lettuce homogenate, however, sanitizer efficacy was reduced. Exposure to 25 and 100 ppm sanitizer at 10°C for 60 s inactivated MNV-1 by an average 1.42-$\log_{10}$ and 1.67-$\log_{10}$ PFU/ml, respectively. At 20°C, MNV-1 was inactivated by an average 0.43–$\log_{10}$ and 1.44-$\log_{10}$ PFU/ml after 60 s exposure to 25 and 100 ppm sanitizer, respectively. Tailing was observed in inactivation curves, indicating the effectiveness of the sanitizer was greatly reduced after only 15 s by the organics in the lettuce homogenate.

Significance: The data highlight the difficulty in inactivating MNV-1 in the presence of organic matter with sodium hypochlorite at concentrations typical of those used in the fresh and fresh-cut produce industry.

P5-01 DSC Prevalence of Antibiotic-Resistant Bacteria in Deli and Restaurant Foods

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Introduction: The rapid emergence of antibiotic-resistant (ART) pathogens represents a significant risk to public health. Commensal bacteria likely play a critical role in the dissemination of antibiotic resistance (AR) genes within the microbial ecosystems, and to pathogens. Recent findings on the prevalence of ART commensal bacteria in retail food products suggest that the food chain may have become an important avenue of transmitting AR to humans.

Purpose: The objective of this study is to investigate the contribution of deli and restaurant foods to the transmission of AR to humans.

Methods: Food samples were obtained from randomly selected salad bars in supermarkets and restaurants in the Columbus, OH area, and subjected to assessment for ART bacteria by use of BHI-Tet plates. The presence of Tetr-encoding genes was examined by PCR. The plasmid profiles of resistant isolates were analyzed. AR gene carriers were identified by partial 16S rRNA gene sequence analysis.

Results: ART bacteria were detected from 7 out of the 8 salad samples containing uncooked vegetables, 3 out of the 4 seafood samples, 3 out of the 4 cheese samples, and all 5 meat and 4 cereal samples examined. The ART bacteria ranged from 10^1 to 10^5 CFU/g of food. ART bacteria counts were lower in cooked food samples than uncooked items. Seventy out of 1069 isolates from BHI-Tet plates contained tetM genes. Different plasmid profiles were found in 18 ART isolates examined. Out of 14 tetM+ isolates examined, 12 grew in media containing 128 µg/ml Tet. So far one *Lactococcus* sp. and three *Enterococcus* sp. isolates have been identified as tetM gene carriers.

Significance: Our results suggest that deli and restaurant foods may also play a role in transmitting AR to humans. Proper processing and management of these foods should be part of the integrated effort against AR transmission to humans.

P5-02 Trends from 2002 through 2006 in Total *Campylobacter* and Total Ciprofloxacin-Resistant *Campylobacter* Loads in Rinses from Retail Raw Broiler Chicken Carcasses

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Introduction: Using a normally lethal dose of ciprofloxacin in a *Campylobacter* agar medium, we have previously published a new method for selective isolation and enumeration of presumptive ciprofloxacin-resistant *Campylobacter* load from total *Campylobacter* load in rinses from retail raw chicken carcasses.

Purpose: Our objective is to determine the long-term quantitative trends in total *Campylobacter* CFU and total ciprofloxacin-resistant *Campylobacter* CFU in retail raw chicken carcass rinses.

Methods: We determined total *Campylobacter* CFU and total ciprofloxacin-resistant *Campylobacter* CFU per carcass over a period of five years from 2002 thorough 2006 by sampling a total of 884 carcasses over 221 weeks at the rate of four carcasses per week. The direct plating detection limit of 0.90 $\log_{10}$ CFU *Campylobacter* carcass was achieved by using centrifuge-pelleted cells from 50/400 ml sub-samples from the carcass rinses. Samples were concurrently spread-plated on *Campylobacter* agar (CA) and on *Campylobacter* agar containing 8.6 µg/ml ciprofloxacin (CCA).

Results: Percentages of carcasses with countable numbers of *Campylobacter* CFU were 86%, 57%, 74%, 96% and 85% for 2002, 2003, 2004, 2005, and 2006 respectively. The total *Campylobacter* load ranges per carcass were 0.90–4.58, 0.90–4.48, 0.90–4.82, 0.90–4.60 and 0.90–4.27 $\log_{10}$ CFU/carcass in 2002, 2003, 2004, 2005, and 2006, respectively. Percentages of carcasses with countable ciprofloxacin-resistant *Campylobacter* CFU/carcass were 60%, 18%, 20%, 42%, and 27% for 2002–2006 respectively. The total

ciprofloxacin-resistant *Campylobacter* load ranges per carcass were 0.90–3.95, 0.90–3.0, 0.90–3.3, 0.90–3.86, and 0.90–3.09 $\log_{10}$ CFU/carcass in 2002–2006, respectively.

Significance: Some reductions were seen for carcasses with higher loads of total *Campylobacter* or total ciprofloxacin-resistant *Campylobacter* during the five year period. However, some colonies of *C. jejuni* randomly picked from carcass rinses directly plated on CA and CCA showed the continued presence of a high degree of ciprofloxacin-resistance, with MIC's ranging from ≥ 16 to ≤ 32 µg/ml.

P5-03 Modeling the Growth of *Listeria monocytogenes* in Delicatessen Turkey and Ham

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Introduction: Several risk assessments indicate that consumption of contaminated delicatessen meat is the leading cause of listeriosis in the United States, accounting for ~1000 of 2500 cases annually, including ~200 fatalities. Therefore, modeling *Listeria monocytogenes* growth in these products is critical if "best consumed by" dates are to be developed.

Purpose: The objectives of this study were to (1) evaluate the validity of the broth-based Pathogen Modeling Program (PMP) for predicting *L. monocytogenes* growth in cured turkey and ham, and (2) model the growth of *L. monocytogenes* in cured turkey and ham as a function of time and temperature, using a model fit specifically to growth data for those products.

Methods: Two brands of ham and cured turkey breast of varying composition were sliced, surface-inoculated with an 8-strain cocktail of *L. monocytogenes* (~40 CFU/g) at the beginning, middle and end of shelf life, and then sampled (25 g) during storage (4, 7, and 10°C) to obtain a series of *L. monocytogenes* growth curves. The resulting data were used to (1) assess the validity of the PMP model in predicting growth of *L. monocytogenes* in the two products and (2) estimate product-specific parameters for a modified Gompertz model.

Results: Overall, the PMP model yielded larger ($P < 0.05$) root mean squared errors (RMSE) for log growth (cured turkey = 3.1; ham = 4.7) compared to the product-specific Gompertz equation (cured turkey = 1.1; ham = 0.9). Plots of PMP-predicted versus observed log counts for *L. monocytogenes* growth showed apparent systematic errors, indicating that the PMP model is inappropriate for modeling *L. monocytogenes* growth in certain cured turkey and ham products.

Significance: The re-parameterized, product-specific Gompertz model, which more reliably estimated *L. monocytogenes* growth in cured turkey and ham, is better suited for predicting "best consumed by" dates for deli meats.

P5-04 Growth Model of a Plasmid-Bearing Virulent Strain of *Yersinia pseudotuberculosis* in Raw Ground Beef

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Introduction: Yersinia pseudotuberculosis (YPST) has been implicated in foodborne illnesses associated with various foods, including raw beef. A 70-kb virulence plasmid (PYV) is involved in expression of virulence. Increased growth temperatures (≥ 30°C) facilitate the loss of this plasmid and the virulence-associated determinants.

Purpose: There are no reports concerning the growth of plasmid-bearing virulent YPST in raw ground beef (RGB). To fully assess the potential risk of illness, it is necessary to know the effect of the endogenous microflora on the behavior/fate of YPST in RGB.

Methods: Ninety-gram portions of retail RGB (~7% fat), irradiated or raw, were artificially contaminated with serotype O:1b YPST PB1/+ strain and rifampicin-resistant-YPST PB1/+ strain (rif-YPST) at a concentration of 10^5 CFU/g. Samples (3-gram) were stored at temperatures ranging from 0–30°C. At various time intervals, samples were serially diluted in 1% peptone water, surface plated onto Congo red-(CR)-magnesium-oxalate agar (CRMOX), and then incubated at 37°C for 48 h for enumeration of pYV-YPST by CR binding. For non-sterile RGB studies, rif-YPST colonies were enumerated on rif-(100 µg/ml)-CRMOX.

Results: In sterile RGB, the growth rate (GR) ranged from 0.0227 to 0.6221 $\log_{10}$ CFU/h at 0 to 25°C and maximum population densities (MPD) ranged from 8.7 to 11.0 log CFU/g. The GR and MPD models were developed as a function of storage temperature. The models were validated with rif-YPST in sterile RGB. In non-sterile RGB, rif-YPST displayed similar GR and MPDs as in sterile RGB. This may be due to low levels (4.31 ± 0.60 log CFU/g) of background microflora in fresh retailed RGB.

Significance: Models for GR and MPD of YPST in RGB displayed acceptable bias and accuracy within 95% of the predicted values. There was eventually no loss of pYV at the temperatures evaluated. Therefore, beef contaminated with YPST could cause disease if the meat was not properly cooked.

P5-05 A Risk Assessment Model of *Vibrio parahaemolyticus* for Consumption of Raw Oysters in Korea

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Introduction: Vibrio parahaemolyticus causes gastroenteritis with severe abdominal pain and diarrhea. Raw fish and shellfish are the main sources of foodborne illness caused by *V. parahaemolyticus*. The outbreak caused by *V. parahaemolyticus* occurs from April to October in Korea.

Purpose: To know the risk caused by *V. parahaemolyticus* by consuming raw oysters in Korea, the retail-to-table pathway was modeled including initial contamination at retail, growth during consumer transport, refrigerator storage and dose-response after consumption.

Methods: The quantitative data of *Vibrio parahaemolyticus* from 133 positive samples of raw Korean oysters according to the seasonal changes from retail outlets was input in this study. The Davey and square root models for the secondary growth models were applied to predict *V. parahaemolyticus* dynamics associated with temperature variables. All the data and the predictive models were used to define the input settings for quantitative microbial risk assessment for *V. parahaemolyticus* in Korean raw oysters with @Risk program. The pathogenicity percentage among *V. parahaemolyticus* and the dose-response model from a US report (2005) were used. The scenario of consumption

pattern and the consumption amount of raw oysters was established by using our consumer survey study (467 people) and the National Health and Nutrition Examination Survey data (2005).

Results: Therefore, when the Korean population over 12 years old, 40,116, 241, was applied to the calculation, the person number daily incidence by pathogenic *V. parahaemolyticus* in raw oysters was estimated at only 2 in April & October (the season for oysters) and 41 from May to September (the off-season).

Significance: This assessment result can be used for risk management programs such as introduction of microbial reduction technology and consumer education.

P5-06 DSC A Model of the Effect of Temperature on the Growth of Pathogenic and Nonpathogenic *Vibrio parahaemolyticus* Isolated from Oysters in Korea

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Introduction: Vibrio parahaemolyticus is recognized as the leading cause of human gastroenteritis associated with the consumption of seafood.

Purpose: The objectives of this study were to compare the growth characteristics of pathogenic strains of *V. parahaemolyticus* that possessed the trh gene and nonpathogenic *V. parahaemolyticus* at various temperatures and to use the data generated by this comparison to model the effect of temperature on lag time and the specific growth rate of *V. parahaemolyticus* in broth and oyster slurry models.

Methods: Primary growth models of *V. parahaemolyticus* in broth and oyster slurry fit well to a modified Gompertz equation (broth R2 = 0.99; oyster slurry R2 = 0.96). The lag time (LT), specific growth rate (SGR), and maximum population density (MPD) of each primary model were compared.

Results: The growth of nonpathogenic *V. parahaemolyticus* was found to be more rapid than that of pathogenic *V. parahaemolyticus*, regardless of the model medium. In addition, significant ($P < 0.05$) differences in the growth kinetics between pathogenic and nonpathogenic *V. parahaemolyticus* in broth were observed at 10°C. When compared to growth in broth, the growth of *V. parahaemolyticus* was delayed in oyster slurry, and growth was not observed at 10°C or 15°C. The Davey and square root models were identified as appropriate secondary models for predicting the LT and SGR, respectively. For the broth model, the average Bf and Af values for LT were found to be 0.97 and 1.3, respectively, whereas the average Bf and Af values for SGR were 1.05 and 1.11, respectively.

Significance: The model generated in this study predicted an LT that was shorter and an SGR that was similar to those that were actually observed, which indicates that these models provide a reliable and safe prediction of *V. parahaemolyticus* growth.

P5-07 A Multi-Factorial Risk Prioritization Framework for Foodborne Pathogens

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Introduction: There is consensus that a science- and risk-based management system to prioritize foodborne hazards is needed. To date, most approaches have been based on measures of health alone.

Purpose: To develop and pilot-test a risk ranking tool that considers public health as well as other criteria to rank foodborne issues.

Methods: Four criteria and 6 food-pathogen combinations, *Campylobacter* in chicken (CC), *Salmonella* in chicken (SC) and spinach (SS), *E. coli* O157 in beef (EB) and spinach (ES), and *L. monocytogenes* in Ready-to-Eat meats (LRTEm), were selected. Public health impact was measured by the cost of illness and disability adjusted life years. Market impact was quantified by the economic importance of the domestic market. Likert-type scales were used to rank consumer perception of risk, and finally, vulnerable consumer group and industries were captured by the social sensitivity criteria. Data from a variety of sources were collected for each of the criteria to build information cards, the first stage of the framework. The data on the information cards were aggregated per food-pathogen combination and a graphical summary profile of the risk was created. Finally, a formal multi-criteria decision analysis using different weights for the 4 criteria was conducted and the criteria were aggregated to produce a ranking of priorities.

Results: Assuming twice as much weight for the public health criteria compared to the others, the final ranking was: 1. CC; 2.EB; 3. SC; 4.LRTEm; 5. ES; and 6.SS. If social sensitivity or consumer perception were removed from the analysis, EB would be ranked 3rd and SC would be 2nd.

Significance: The framework provides a flexible instrument that compares risks along four dimensions. It can be used to establish priorities across pathogens for a particular food, across foods for a particular pathogen and/or across specific food-pathogen combinations.

P5-08 Development of a Logistic Regression Plot for Predicting the Probability of Achieving a 7-Log Reduction of *Escherichia coli* O157:H7 during Beef Slow-Cooking Processes

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Introduction: Slow-cooking processes impart desirable characteristics to whole-muscle beef products. However, the slow temperature increase during these processes may enhance the survival of *Escherichia coli* O157:H7. Government guidance cautions against the use of slow-cooking procedures in which the product temperature increases from 10°C to 54.4°C in > 6 h. Therefore, processors using slow-cooking must prove that their processes adequately destroy *E. coli* O157:H7.

Purpose: The objective of this study was to develop a logistic regression plot of the probability of achieving a 7-log reduction of *E. coli* O157:H7 in beef during slow-cooking.

Methods: Small portions (25 g) of lean ground beef were inoculated (5-strain mixture of anaerobically grown *E. coli* O157:H7) and heated in three trials each of four simulated slow-cooking schedules. Samples taken at nine times per trial were analyzed for log CFU of surviving *E. coli* O157:H7 by an overlay plating method

(overall n = 108). Using a previously determined Z-value (11.4°C) and 54.4°C reference temperature, the cumulative process lethality (F-value) for each sampling time was determined. Logistic regression analysis was used to plot the probability of at least a 7-log reduction of *E. coli* O157:H7 against F-value.

Results: On the logistic regression plot, an F-value of 308 corresponded to a 95% probability of achieving at least a 7-log reduction. This level of reduction was achieved in all 15 samples with F-value of at least 308. Additional ground-beef slow-cooking trials showed that at least a 7-log *E. coli* O157:H7 reduction was consistently achieved in trials with F-value of 308 or higher, suggesting that achieving this F-value was likely to ensure sufficient lethality.

Significance: The logistic-regression-based plot was accurate in predicting whether a 7-log reduction of *E. coli* O157:H7 was achieved in ground beef during slow-cooking. This plot will serve as a basis for evaluating slow-cooking processes used for whole-muscle beef products.

P5-09 Comparison of Three Mathematical Approaches to Predicting Pathogen Growth During Short-Term Temperature Abuse of Raw Meat and Poultry Products

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Introduction: Different mathematical approaches exist for calculating pathogen lag-phase duration (LPD) and growth rate (GR).

Purpose: We compared three approaches for calculating LPD and/or GR values for use in tools to predict growth of *Salmonella* serovars, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in temperature-abused raw meat and poultry.

Methods: Using log CFU/g data from small-scale (25 g) isothermal experiments conducted with bratwurst, ground beef, and ground turkey, DMFit software determined LPD and GR at specified temperatures from 10 to 46.1°C. Linear interpolation of these values was used to develop an interval accumulation-based tool (THERM v.2). A second interval-accumulation-based tool, THERM, v.3, used quadratic equations describing the LPD and GR values, with a minimum LPD of the lowest experimentally-derived value, and a minimum GR of zero. Development of THERM v. 4 involved the THERM v. 3 LPD calculations and GR calculated from linear interpolation of square root (generations/h) vs. temperature. Time/temperature and pathogen log CFU/g data were obtained from 9 inoculation experiments with ground beef, intact and ground poultry, and pork sausage that resulted in pathogen growth of 0 to 5.4 log CFU. The time/temperature history from each experiment was entered into all three tools to obtain pathogen growth predictions.

Results: When predicted and experimental results were described as growth (> 0.3 log increase in CFU) or no growth, all three tools yielded 23 accurate predictions and one fail-safe prediction out of 24 pathogen/product combinations. When observed Δlog CFU values were compared with predicted values, the three tools had accurate + fail-safe rates of 83%, but THERM v. 2 was most often accurate (46% vs. 25% for THERM v. 3 and v. 4).

Significance: Linear interpolation of LPD and GR values determined from experimental data and DMFit is an acceptable approach in developing interval-accumulation-based tools for predicting pathogen growth in raw meat and poultry.

P5-10 Fuzzy Math Calculation for Quantitative Risk Assessment of Roasted Duck Cuisine

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Introduction: Fuzzy sets, as possibility distributions, offer a powerful tool for the modeling of various fields. A Monte Carlo simulation was used to predict the contamination levels of a microorganism. This study was carried out to develop a quantitative risk assessment model for *Staphylococcus aureus* in roasted duck on the menu of a French restaurant in Seoul (Korea) and establish the specification for its risk management. The fuzzy math calculation was compared with a Monte Carlo simulation in terms of the propagation of uncertainty.

Purpose: The purpose of this study was to evaluate the use of fuzzy math calculation to calculate variability and uncertainty in quantitative risk assessment of a French main dish such as roasted duck fillet with bigarad sauce.

Methods: Exposure assessment of duck was performed in four steps: warehousing duck and vegetables (raw material), pretreatment, cooking and dining. In the fuzzy math calculation, fuzzy values of the microbial data were established and á-cut method was applied to the further operations. To conduct the Monte Carlo simulation, Palisade's @RISK software (Palisade Corp., Newfield, NY, USA) was used as an add-in to Microsoft Excel. Each simulation was run with 10,000 iterations, using Latin-Hypercube sampling.

Results: In the duck dish, the minimum and maximum values for risk obtained with fuzzy math were 0.47 log CFU/g and 6.79 log CFU/g, and those of the Monte Carlo simulation were 0.54 log CFU/g and 3.31 log CFU/g, respectively. The mean values for the risk from fuzzy math and Monte Carlo simulation were 2.14 log CFU/g and 1.54 log CFU/g, respectively. The distribution of values obtained using the fuzzy math simulation included all of the results obtained using the Monte Carlo simulation. The estimates resulted from fuzzy math calculation were similar to those of the Monte Carlo simulation in terms of accuracy. However, variability of fuzzy estimates was larger and calculation load was smaller compared with those of the Monte Carlo simulation.

Significance: Fuzzy math simulation was found to be a good alternative to the Monte Carlo simulation in quantitative risk assessment of a duck dish.

P5-11 Development of a Predictive Model of *Staphylococcus aureus* as a Function of Storage Temperature, pH, and Concentration of NaCl

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Introduction: Predictive food microbiology (PFM) is an emerging area of food microbiology. It applies mathematical models to predict the response of microorganisms to specified environmental variables. Although at present PFM models are not completely developed,

models can provide very useful information for microbiological responses in HACCP (Hazard Analysis Critical Control Point) systems and Risk Assessment.

Purpose: The purpose of this study was to develop a reparameterized Gompertz model of *S. aureus* in imitation crab sticks as a function of storage temperature, pH, and concentration of sodium chloride (NaCl).

Methods: A full-factorial design was applied in this study, using three levels of each factor. Growth curves of *S. aureus* were obtained by measuring cell concentration in culture broth under different conditions (storage temperature, 5°C, 20°C, and 30°C; pH 4.0, 5.5, and 7.0; NaCl content levels 1.0%, 4.0% and 7.0%). The growth curves were used to establish a Gompertz model of *S. aureus*. Gompertz values such as A, C, B, and M, and growth kinetics such as exponential growth rate (EGR), generation time (GT), lag phase duration (LPD), and maximum population density (MPD) were calculated based on growth data.

Results: GT and LPD values gradually decreased, whereas EGR value gradually increased with increasing temperature. Response surface analysis (RSA) was carried out using Gompertz B and M values, to formulate an equation using temperature as a main control factor. This polynomial equation was applied to the Gompertz equation. Experimental and predictive values obtained from the reparameterized Gompertz model showed no significant differences ($P < 0.01$).

Significance: This proposed model could be used to predict growth of microorganisms for exposure assessment of MRA (Microbial Risk Assessment). In addition, this model will provide us with more informed decision making on potential regulatory actions related to microorganisms in imitation crab sticks.

P5-12 Risk Assessment of Non-Heat Preparation of Japanese Foods, Using Quantitative Risk Assessment

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Introduction: Quantitative Risk Assessment (QRA) is the method for calculating numerical risk levels for comparison with regulatory risk criteria.

Purpose: We used Monte Carlo Simulation (MCS) to predict contamination levels of *Staphylococcus aureus* on the raw materials, equipment and cook in a Japanese restaurant located in Seoul and identified hazardous factors.

Methods: Fresh salad vegetables such as cabbage, cucumber, hot pepper, unripe beans, and sushi such as shrimp and octopus were purchased from a Japanese restaurant in Seoul. The environment was tested on the cook's hand, knife, chopping board, mixing bowl and dish. *S. aureus* on raw material and environment were detected by Baird Paker RPF medium. To conduct a Monte Carlo simulation, Palisade's @RISK software (Palisade Corp., Newfield, NY, USA) was used as an add-into Microsoft Excel. Each simulation was run with 10,000 iterations, using Latin-Hypercubesampling

Results: 'Serving temperature' had the highest sensitivity value, 0.793, among factors of fresh salads by sensitivity analysis. In shrimp and octopus sushi, 'serving temperature' also had the highest sensitivity value, 0.419, followed by 'storage time', 0.374. To increase safety of sushi, consumers should consume sushi as soon as possible after preparation. In sushi 'storage time after cooking' was determined as CCP. To determine the Critical Limit (CL), Scenario Analysis (SA) was carried out. In sushi, SA was carried out using serving time as a unit condition. The safety level of *S. aureus* was set lower than 5 log CFU/g according to Korean Food Code. In the case of fresh salads, 'frequency of washing' was the unit condition for SA. The number of *S. aureus* in salads was reduced significantly by washing. Since processes for preparing fresh salads were less than the other Japanese restaurant menu items, 'frequency of washing' should be set higher than the others.

Significance: Based on this study, QRA was a beneficial tool for evaluating factors influencing potential risk and could be applied directly to risk management.

P5-13 DSC Survival of *Staphylococcus aureus* in Salsa Mexicana

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Introduction: Mexican food is often implicated in outbreaks of foodborne illness, but limited safety information is available. Salsa, a common Mexican side dish, requires considerable handling during preparation. It is frequently served at slightly elevated temperatures and often involved in staphylococcal food poisoning.

Purpose: This study evaluated the effects of storage temperature on the survival of *Staphylococcus aureus* in commercial Mexican salsa.

Methods: Restaurant salsa samples were inoculated with a cocktail suspension of *Staphylococcus aureus* strains at either 1,550 cells/g (high inoculation level) or 155 cells/g (low inoculation level). *Staphylococcus aureus* populations (CFU/g) were determined using 3M Petrifilm™ Staph Express Count Plate at 2, 4, 6 and 24 h of storage at 25°C and 1, 3, 5, and 7 days of storage at 4°C.

Results: A decline in *Staphylococcus aureus* populations was observed ($P < 0.0001$) at both storage temperatures and inoculation levels. For samples stored at 25°C, although the microbial numbers declined significantly at 24 h, the numbers for both low and high inoculation levels remained high during the first 6 h. This result reveals the potential for risk if salsa is contaminated during preparation. For samples stored at 4°C, a similar trend was observed. The greatest reduction occurred after 7 days of storage for both high and low inoculation levels (2.51 log CFU/g and 1.79 log CFU/g, respectively). However, the microbial load remained high during the first 3 days at 4°C, suggesting that *Staphylococcus aureus* will persist in salsa once it is contaminated.

Significance: These results demonstrate that despite the high acidity levels typically found in salsa, *Staphylococcus aureus* populations can persist during typical consumption time frames. The results reveal the importance of proper handling and storage of salsa both during and after preparation

P5-14 Comparison of Linear vs. Non-Linear Models to Describe the Thermal Inactivation Kinetics of Heat-Resistant *Bacillus* Spores

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Introduction: Linear trend models have been used extensively by the food industry as a guideline for the safe processing of food products. Due to the influence of environmental factors, such as biological strain varia-

tions and microbe-microbe interactions, the inactivation kinetics may tend to be non-linear. Under such conditions, if first-order inactivation kinetics are assumed and D_{10}-values used, erroneous conclusions may be drawn.

Purpose: The purpose of this study is to evaluate and compare linear vs. non-linear models to estimate the survival of heat resistant *Bacillus* spores subjected to thermal processing conditions similar to those in the food industry.

Methods: Bacillus sp. spore suspensions in capillary tubes were subjected to 115, 120, 125°C in an oil-bath. The capillary tubes were removed from the oil-bath at 60, 75, 90, 120, and 150 s and survivors were enumerated on Dextrose Tryptone Agar. Plates were incubated at 35°C for up to 72 h. Linear survivor curves were generated using least-squares regression and D-values were calculated by taking the negative reciprocal of the survivor curve slope. Non-linear survivor curves were generated by fitting quadratic models to the data. Data analysis was accomplished using SAS software.

Results: The inactivation kinetics for the evaluated *Bacillus* spp. spores followed a non-linear trend. The variation in the experimental data matched more closely with quadratic models than with linear models. The R-square value for the linear models ranged from 0.46 to 0.86 whereas the R-square value for the non-linear models ranged from 0.73 to 0.99.

Significance: In this study, quadratic models were found to be more appropriate in quantifying the survival of heat-resistant *Bacillus* spores than linear regression models. Food processors should rely on alternative approaches when log-linear models fail first-order kinetics to ensure microbiologically safe products.

P5-15 DSC Development of a Mathematical Model for Growth of *Salmonella* on Cut Tomatoes

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Introduction: At least five outbreaks of salmonellosis associated with tomatoes have occurred since 2000. The cause of these outbreaks appears to be contamination originating during agricultural production, but the exact source is unknown.

Purpose: It is known that cut or sliced tomatoes that are unrefrigerated will support the growth of *Salmonella*. This research was initiated to develop a mathematical model capable of predicting the growth rate of *Salmonella* in tomatoes as a function of incubation temperature.

Methods: Four different human isolates of *Salmonella* epidemiologically linked to consumption of tomatoes were obtained from the CDC. Whole tomatoes were dip-inoculated using a cocktail of the four strains and dried overnight. Inoculated tomatoes were cut with a sterile knife, placed in a plastic bag and incubated in a temperature-controlled water bath at 15, 20, 25 or 30°C. Growth of *Salmonella* was monitored by plating on XLT4 agar. Growth rates were calculated using the Baranyi model, and a variety of secondary models were used to predict growth rate as a function of temperature.

Results: Salmonella growth rates increased with increasing temperature. An acceptable correlation was seen when predicting the square root of the growth rate vs. temperature (R2 = 0.8613), but a distinct non-linear trend was seen in the data. Changing the transformation to the natural log of growth rate increased the correlation (R2 = 0.9295), and using the inverse of growth rate resulted in the best correlation (R2 = 0.9518); the latter choice also eliminated the non-linearity.

Significance: Although salmonellosis associated with tomatoes will remain a concern until the ultimate source of *Salmonella* bacteria can be identified and eliminated, our mathematical model provides a useful tool for estimating the risk posed by different abuse temperatures. This model may also be useful in the development of quantitative risk assessment for *Salmonella* in cut tomatoes.

P5-16 Assessment of Microbiological Quality in Korean Traditionally Fermented Jeot-gal Products

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Introduction: Jeot-gal, a traditional Ready-to-Eat fermented fish product, is widely consumed in Korea. Salts and spices are added during the preparation. Many products commercially available in the marketplace are a mixture of seasoned and fermented Jeot-gal and some kinds of spices. The Korea Food Code designates Jeot-gal products as category 5. 24.

Purpose: The Korea Food Code describes microbiological limitations on fish sauce only. This study was performed to establish baseline and provide guidelines of hygienic process control, and to suggest effective microbial standards for Jeot-gal products.

Methods: A total of 554 Jeot-gal samples were analyzed for coliform and *E. coli* population levels, as well as presence of *Staphylococcus aureus* and *Vibrio parahaemolyticus*. All microbial methods were based on the Korea Food Code.

Results: Total coliform detection rates of Jeot-gal and seasoned Jeot-gal were 6.0% and 37.7%, respectively. Seasoned Jeot-gal demonstrated 4–6 fold higher coliform levels than Jeot-gal. *E. coli* was detected at an average rate of 1.6% in only in seasoned Jeot-gal. *E. coli* was detected primarily from products containing below 10% NaCl. *S. aureus* and *V. parahemolyticus* were not detected in any samples.

Significance: There is a trend towards low sodium Jeot-gal products. Data from this study will help to characterize the baseline hygenic conditions during processing and help define potential microbial guidelines in Korea for this product group.

P5-17 Microbiological Quality of Food in the State of Hidalgo, México

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Introduction: In Hidalgo, as in many states, the consumption of food prepared outside the home has increased significantly in recent years. There is currently little information available on the microbiological quality of food in Hidalgo. In response to this need, the State Laboratory of Public Health (LESPH) in coordination with the Commission for Protection Against Health Risks conducted microbiological analysis of different foods.

Purpose: The purpose of the study was to determine the microbiological quality of the foods that are offered for sale in Hidalgo.

Methods: Samples were received at State Laboratory, from 12 jurisdictions, through a surveillance program administered in coordination with Sanitary Authorities. Almost three thousand samples (n = 2,777) of foods, including dairy products, ready-to-eat foods, meats, vegetables, fish and seafood, were processed during the period January 2006 to August 2007. The determinations were made for aerobic mesophilic bacteria, total and/or fecal coliforms, molds and yeasts, *Staphylococcus aureus*, or *Salmonella* spp., using Mexican Official Standards techniques.

Results: Approximately 15% of the 2,419 samples analyzed for aerobic mesophilic bacteria, total and/or fecal coliforms, molds and yeasts were found to have excessively high counts. A total of 358 fish and shellfish samples were tested. *Vibrio cholerae* NE 01, *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. fluvialis*, *V. mimicus* and *V. vulnificus* were isolated in 88, 29, 5, 4, 2, and 1 sample, respectively. A significant number of cheese samples (86/358) were found to have *S. aureus* counts above that allowed by Mexican standards. *Salmonella* spp. were isolated in 18 of 168 samples of raw and processed meats.

Significance: The results show that inadequate hygienic practices exist in the preparation or food preservation in the State of Hidalgo, and that improvement is still needed. It is important to note that the health controls implemented by the Commission for Protection Against Health Risks State have improved the sanitary quality of food when compared to data from 2002, when 56% of foods tested were found to be out of compliance.

P5-18 Groups Unaware of Food Recall: Policy Implication

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Introduction: In September of 2006, the Food and Drug Administration issued an advisory warning against the consumption of fresh spinach because of contamination with *E. coli* O157:H7; spinach was largely recalled by manufacturers. Messages of the recall were communicated by regulatory agencies and manufacturers to the public via the media.

Purpose: The purpose of this study was to examine which groups of people are most likely to be uninformed about food recalls.

Methods: Using Computer Assisted Telephone Interviews (CATI), a nationally representative sample of 1,200 Americans over age 18 was interviewed in November, 2006. CATI programming ensured an equal number of male and female participants were surveyed.

Results: Eighty-seven percent of all participants were aware of the spinach recall. In order to explore which segments of the population were unaware of the spinach recall, two regression models were used. The first model included the following demographicvariables: gender, ethnicity, and age. Persons most likely to be unaware of the recall were those who were male, of African American or Hispanic decent, or under age 35 (R2 = 0.06). The second regression analysis (R2 = 0.01) showed that not being the primary care giver of a child and having a child under 18 in the home was predictive of being unaware of the spinach recall.

Significance: Persons who were unaware of the spinach recall could be more likely to be unaware of future food recalls. Now that groups likely to be unaware of recalls have been identified, better efforts, using appropriate techniques, should be utilized to reach these segments of the population. This can help to increase the responsiveness of the public to food recalls, and in addition may help prevent sickness and death resulting from unawareness of food recalls.

P5-19 Profile of the Temperature of Meals Consumed by Sugar Cane Cutters

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Introduction: In Brazil, approximately 75% of the sugar cane is cut manually by rural workers known as sugar cane cutters. They bring from home their meals to be eaten at lunch time. In the fields they have no place to store their meals until the moment of consumption and since there is no control of the temperature there is a great potential for growth of microorganisms that may cause foodborne disease.

Purpose: The purpose was to check if the conditions of temperature and storage time of the meals consumed by the sugar cane cutters are within the danger zone in terms of microbial growth.

Methods: The meal temperature was measured at the time of arrival at the farm and at the time of consumption. Also, the temperature was taken in between the two measurements.

Results: Thirty-nine meals a day were analyzed, two days a week during five weeks, for a total of 10 days. Data collection was performed in a sugar cane plantation located in the state of São Paulo, Brazil. The average temperature of the meals at time of arrival at the farm was 49.2°C (± 2.1°C) and they were consumed after an average of 222 minutes. The average temperature at the time of consumption was 36.1°C (± 3.2°C). The data show that the meals remained in the danger zone for an extended period of time prior to consumption. Such practice has great possibility of contamination and growth of microorganisms, putting the health of the workers at risk.

Significance: The results showed the need for sugar cane cutters to have both a suitable container and place for the maintenance of temperature of their meals to prevent possible deterioration, with health consequences to the employees.

P5-20 Risk Assessment of Lettuce Contamination with *Escherichia coli* O157:H7

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Introduction: Recent outbreaks of foodborne disease associated with the consumption of fresh produce raised concern that these products may be a source of pathogens such as *E. coli* O157:H7. Currently, contamination risks have not been quantified.

Purpose: The purpose of this study was to construct a quantitative probabilistic model to estimate the likelihood of lettuce contamination with *E. coli* O157:H7 from manure-amended soil. In addition, the effects of intervention strategies were assessed.

Methods: The transmission of *E. coli* O157:H7 was modeled by following probability distributions of pathogen prevalence and density along different modules: cattle, manure, manure-amended soil and lettuce. Parameter values were based on published data and

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experiments. The model was constructed in Microsoft Excel using @Risk software (version 4.5.5, 2004, Palisade Corporation). Importance and sensitivity analysis was conducted to identify the most important predictive factors and to assess the efficiency of risk mitigation strategies. A traditional organic and an intensive conventional scenario were compared.

Results: The model estimated an average of 0.34 contaminated lettuce plants per hectare. No confidence limits or uncertainty bounds could be associated with the expected probabilities because they represent both uncertainty and variability. The estimated number of contaminated heads was most sensitive to the prevalence of contaminated manure, the manure storage time and the initial density of *E. coli* O157:H7. Testing these hypothetical intervention strategies with data revealed that the use of a minimum manure storage time of 30 days and a minimum fertilization-to-planting interval of 60 days were most successful. A traditional organic production scenario resulted in a decrease to 0.10 contaminated plants per hectare, while an intensive conventional scenario resulted in an increase to 0.89 contaminated plants per hectare.

Significance: The current model is a first attempt to quantify the risk of lettuce contamination from manure-amended soil and can be used to assess the effects of intervention strategies.

P5-21 Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* in Garlic Powder and Onion Powder

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Introduction: Onion and garlic contain allicin, an antimicrobial compound. When onion and garlic are dried and ground to a powder, the antimicrobial activity of allicin is limited; therefore the survival of vegetative bacteria entering the product through post-process handling is of concern.

Purpose: This study investigated the survival of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in onion and garlic powder stored at room temperature.

Methods: Bacterial cultures resistant to rifampicin were individually cultivated in BHI broth supplemented with 200 ppm rifampicin. Inocula cocktails were prepared with equal volumes of each pathogen and washed by centrifugation. Ten ml of each cocktail was added to 30 g sterile sand in Petri dishes and allowed to dry for 24 h. Two grams of inoculated sand were added to five replicate Whirl-Pak bags containing 200 g of onion or garlic powder, mixed thoroughly, and incubated at room temperature. Target inoculation levels were 4–5 log CFU/g. On days 0, 7, 14, 28, 56, 84, and 120, twenty-five g allquots of inoculated onion or garlic powder were analyzed by pour-plate, using TSA supplemented with 200 ppm rifampicin.

Results: The test organisms in onion and garlic samples were not completely inactivated within 120 days of storage. In onion samples, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* levels decreased significantly ($P < 0.05$) by 1.53 log CFU/g, 3.22 log CFU/g, and 1.27 log CFU/g, respectively. In garlic samples, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* levels decreased significantly ($P < 0.05$) by 1.94 log CFU/g, 3.10 log CFU/g, and 2.01 log CFU/g, respectively.

Significance: These data suggest that *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* can survive for long periods of time in onion and garlic powder stored at room temperature, although their concentrations decline gradually.

P5-22 Edible Apple Film Wraps Containing Plant Antimicrobials Inactivate *Salmonella enterica* and *Escherichia coli* O157:H7 on Poultry

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Introduction: Present day consumers prefer natural products over synthetic additives. Compounds derived from plant sources have shown antimicrobial activity against a number of foodborne bacteria.

Purpose: As part of an effort to discover new ways to improve microbial food safety, the objective of the present investigation was to evaluate apple based edible films containing plant antimicrobial compounds for their activity against pathogenic foodborne bacteria on raw poultry.

Methods: Salmonella enterica or *Escherichia coli* O157:H7 (10^5 CFU/g) cultures were surface inoculated on chicken breasts. The poultry products were then wrapped with apple based edible films containing three concentrations (0.5, 1.5 and 3%) of cinnamaldehyde (the main ingredient of cinnamon oil) or carvacrol (the main ingredient of oregano oil). Following incubation at either room (21°C) or refrigeration (4°C) temperature for 72 h, samples were stomached in BPW, diluted, and plated for enumeration of survivors.

Results: The results showed that the antimicrobial films exhibited strong, concentration-dependent activities against *S. enterica* and *E. coli* O157:H7 at both temperatures. At room temperature, films with 3% antimicrobials showed the highest reductions (5–7 log CFU/g) of both *S. enterica* and *E. coli* O157:H7 compared to control samples. Films with 1.5% and 0.5% antimicrobials showed 3–5 and 2–3 log CFU/g reductions, respectively. At 4°C, carvacrol exhibited stronger activity than cinnamaldehyde; films with 3, 1.5 and 0.5% carvacrol reduced the bacterial populations by 3, 2.5–3, and 0.6–1 log CFU/g, respectively. Films with 1.5% cinnamaldehyde induced 1–2 log CFU/g reductions. Very little reduction (0.5 log CFU/g) was seen with 0.5% cinnamaldehyde.

Significance: This study demonstrates the potential of edible apple films containing antimicrobials to inactivate pathogenic bacteria on contaminated poultry, and possibly other contaminated meat products, as well as produce. The results suggest that the food industry and consumers could potentially use these films as wrappings to control surface contamination by pathogenic microorganisms.

P5-23 Antimicrobial Activities of Cinnamaldehyde and Carvacrol against Antibiotic-Resistant *Campylobacter jejuni* and *Salmonella enterica* Strains

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Introduction: Consumers generally prefer natural compounds to synthetic additives. Compounds derived from plant sources such as essential oils, their active components, spices and plant extracts have been

shown to exhibit antimicrobial effects against a number of foodborne pathogens.

Purpose: The objective of the present study was to define the antimicrobial activities of two such compounds, cinnamaldehyde (the main ingredient of cinnamon oil) and carvacrol (the main ingredient of oregano oil), against antibiotic resistant strains of *Campylobacter jejuni* and *Salmonella enterica.*

Methods: Different concentrations in sterile phosphate buffered saline (0.05, 0.1 and 0.2% v/v for *Campylobacter*, and 0.1, 0.2 and 0.3% for *Salmonella*) of cinnamaldehyde and carvacrol were added to cultures with initial populations of 10^4 CFU/ml. The samples were then mixed and incubated at 37°C. Viable populations were enumerated at various time points (0, 30, 60 and 120 min for *Campylobacter* and 0, 1, 5 and 24 h for *Salmonella*).

Results: The results indicate that the extent of inhibition of microbial survival was related to both the nature and concentration of antimicrobials and the incubation time. Both cinnamaldehyde and carvacrol showed complete inactivation at 0.3 and 0.4% concentrations for *Salmonella* and at 0.2% for *Campylobacter* at all time points tested. The antimicrobial efficacy of cinnamaldehyde was greater than that of carvacrol. Cinnamaldehyde at concentrations > 0.1% and carvacrol at 0.2% exhibited rapid activity at 120 min against both antibiotic-resistant and nonresistant strains of *Campylobacter.* For *Salmonella* strains, cinnamaldehyde at concentrations > 0.1% completely inactivated some strains. Similar results were obtained with carvacrol at concentrations of 0.1%– 0.3% against resistant and nonresistant *Salmonella* isolates.

Significance: Because resistant and nonresistant strains of the pathogens showed similar susceptibilities to inactivation, these studies provide candidates for incorporation into formulations, safe and effective antimicrobial plant compounds that can concurrently reduce antibiotic-resistant and non-resistant pathogens in animal feeds and human foods.

P5-24 The Use of Caseicin Antimicrobial Peptides for the Inhibition of *Escherichia coli* O157:H7

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Introduction: E. coli O157:H7 is a foodborne pathogen that causes serious illness in humans. Reducing the risk of contamination and transfer of *E. coli* O157:H7 from animals to food can reduce the risk of infection. Investigations into the potential use of antimicrobial peptides (AMPs) to control *E. coli* O157:H7 at key points in the beef chain is ongoing.

Purpose: The purpose of this study was to evaluate the activity of AMPs caseicin A and B (Produced by *Lactobacillus acidophilus* DPC6026 fermentation of sodium caseinate) for the inhibition of *E. coli* O157:H7 in a broth system at 37°C.

Methods: Caseicin A and B were tested against *E. coli* O157:H7 (380-94) and *E. coli* (JM109). Minimum inhibitory concentration (MIC) values were determined via a microtitre plate method. Strains were exposed to various concentrations (0.004–2 mg/ml) of each AMP in diluted (1:10) Tryptic Soy Broth at 37°C for 16–18 h. The MIC was defined as the lowest concentration of AMP that resulted in no visible growth after 18 h. Experiments to determine number of surviving *E. coli* following exposure to each AMP (at their respective MIC values) over 24 h were performed. All cell counts were compared by use of ANOVA.

Results: For caseicin A and B, the MIC values for *E. coli* O157:H7 were 1 mg/ml and 2 mg/ml, respectively. Inhibition of *E. coli* O157:H7 and *E. coli* JM109 did not significantly ($P > 0.05$) differ for caseicin A or B. The numbers of surviving *E. coli* O157:H7 in the presence of caseicin A (1 mg/ml) and B (2 mg/ml) was significantly ($P < 0.05$) lower (4.7–5.0 log CFU/ml) than in the control cultures (no peptide).

Significance: The data suggests that caseicin A and B can inhibit *E. coli* O157:H7 in a broth system at 37°C. Further work involving different environmental parameters is required to determine whether these peptides could be used in food systems.

P5-25 Biocontrol of *Escherichia coli* O157:H7 on Fresh-Cut Lettuce and Cantaloupe by Treatment with Bacteriophage

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Introduction: Outbreaks of foodborne illness have been associated with the consumption of cantaloupes and fresh-cut lettuce. Bacteriophage mixtures may be effective biocontrol agents to reduce *E. coli* O157:H7 on produce.

Purpose: The effectiveness of a mixture of bacteriophages (ECP-100) in reducing populations of *E. coli* O157:H7 on cut pieces of iceberg lettuce and cantaloupe was determined.

Methods: E. coli O157:H7 gfp 86 was spot inoculated on lettuce pieces (9 cm^2) with 3.76 log CFU/cm^2, allowed to dry for 1 h, and then sprayed with either control (phosphate buffered saline) or ECP-100 to deliver 7.98 log PFU/cm^2 before being stored for either 0, 1, or 2 days at 4°C. Cut pieces of cantaloupe were spot inoculated with *E. coli* O157:H7 (4.55 log CFU/ml) and treated with control or ECP-100 (6.69 log PFU/ml), and then stored at 4 or 20°C for 0, 2, 5, or 7 days. On appropriate days, lettuce and cantaloupe samples were homogenized and populations of *E. coli* O157:H7 were enumerated on Sorbitol MacConkey Agar with ampicillin. Significant ($P < 0.05$) effect of phage treatment on bacterial populations was determined.

Results: Populations of *E. coli* O157:H7 on lettuce treated with ECP-100 on 0, 1, and 2 days (0.72, < 0.22, and 0.58 log CFU/cm^2 of lettuce) and stored at 4°C were significantly lower than on those treated with control (2.64, 1.79, and 2.22 log CFU/cm^2), respectively. Populations on cut cantaloupes treated with ECP-100 on days 2, 5, and 7 (0.77, 1.28, and 0.96 log CFU/ml) and stored at 4°C were significantly lower than on those treated with control (3.34, 3.23, and 4.09 log CFU/ml), respectively.

Significance: The bacteriophage treatment reduced populations of *E. coli* O157:H7 immediately upon application to lettuce. This study is the first to show the effectiveness of a bacteriophage mixture to kill *E. coli* O157:H7 on fresh-cut lettuce and cantaloupes.

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P5-26 Mechanism of Inactivating *Escherichia coli* O157:H7 by Ultra-High Pressure in Combination with Tert-Butylhydroquinone

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Introduction: *Escherichia coli* O157:H7 is a significant foodborne pathogen. The pathogen may develop resistance to food preservation methods, particularly those relying on ultra-high pressure (UHP) and other emerging technologies. Therefore, scientific and industrial groups are in search of agents that synergistically enhance the efficacy of these emerging technologies; safety of 'food of the future' depends of the success of these efforts.

Purpose: This study investigated the mechanism of sensitizing *Escherichia coli* O157:H7 and selected *E. coli* mutant strains to UHP by tert-butylhydroquinone (TBHQ).

Methods: *Escherichia coli* O157:H7 EDL-933 and fourteen *E. coli* K12 strains with mutations in selected genes were tested. All strains were grown to stationary phase (~1.0×10^9 CFU/ml) in Tryptose Broth. TBHQ (15–30 ppm), dissolved in dimethyl sulfoxide (DMSO), was added to the culture in a sterile stomacher bag and processed with UHP (400 MPa, 23 ± 2°C for 5 min). After pressure treatments, samples were serially diluted with 0.1% peptone water, plated on Tryptose Agar and incubated at 35°C for 48 h to enumerate the survivors.

Results: Treatment with UHP alone decreased populations of wild-type *E. coli* strains by 2.4–cm² 3.7 log CFU/ml, whereas presence of TBHQ increased UHP lethality by 1.1–6.0 log CFU/ml; TBHQ without pressure was minimally lethal. Response of *E. coli* K12 mutants to these treatments suggests that iron-sulfur-cluster containing proteins ([Fe-S]-proteins), particularly those related to the sulfur mobilization (SUF system), nitrate metabolism, and intracellular redox potential, are critical to the UHP-TBHQ synergy against *E. coli*. Mutations in genes maintaining redox homeostasis and anaerobic metabolism were associated with UHP-TBHQ resistance.

Significance: This study suggests that the redox cycling activity of cellular [Fe-S]-proteins may oxidize TBHQ, potentially leading to the generation of bactericidal reactive oxygen species. Results may ultimately provide guidance to food processors using UHP-based preservation.

P5-27 The Probability of Growth of *Listeria monocytogenes* in Minced Salmon and Tryptic Soy Broth Containing Salt and Phenol Compounds during Storage at Various Temperatures

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Introduction: Smoked salmon is a Ready-to-Eat product. If not processed properly, smoked salmon could be contaminated with *Listeria monocytogenes*.

Purpose: This study modeled the probability of growth of *L. monocytogenes* in minced cooked salmon and Tryptic Soy Broth (TSB) added with salt and liquid smoke (phenol compounds) at various storage temperatures, and explored the possibility of using TSB as a model system for studying the fate of *L. monocytogenes* in smoked salmon.

Methods: A six-strain mixture of *L. monocytogenes* was separately inoculated into cooked salmon and TSB with added 0–10% NaCl and 0–34 ppm phenol to an inoculum of 2–3 $\log_{10}$ CFU/g. Samples were vacuum-packed and stored at 0–25°C for up to 42 days. A total of 32 treatments, each with 16 samples, were tested. A logistic regression was used to model the probability of growth of *L. monocytogenes* in salmon and TSB as a function of concentrations of salt and phenol, and storage temperature.

Results: The growth probabilities of *L. monocytogenes* in salmon and TSB decreased with increased concentrations of salt and/or phenol in salmon and TSB, and at lower storage temperatures. The probabilities were affected more significantly by salt and storage temperature than by phenol in salmon and TSB, and were estimated as > 5% higher by TSB model than by salmon model at the same salt/phenol concentrations at storage temperatures < 12°C.

This indicated that the use of TSB as a model system for salmon in examining growth behavior of *L. monocytogenes* may be suitable only at storage temperatures > 12°C.

Significance: The model for salmon may assist the food industry to identify concentrations of salt and phenol and storage temperatures that minimize the probability of growth of *L. monocytogenes* in smoked salmon. The possibility of using TSB as a model system for salmon provides a simple approach to examining growth behavior of *L. monocytogenes* in smoked salmon.

P5-28 Predictive Modeling of *Listeria monocytogenes* on Cured and Uncured Turkey Breast for Safety-Based Shelf-Life Determination

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Introduction: Contamination of Ready-to-Eat poultry products with *Listeria monocytogenes* has been recognized as an important public health issue. Mathematical models predicting growth of the pathogen during product storage and distribution can be useful in determining safety-based product shelf life under different conditions, assuming that the product is contaminated.

Purpose: The aim of this work was to develop a predictive model for the growth kinetics of *L. monocytogenes* on Ready-to-Eat cured and uncured turkey breast products.

Methods: Commercially manufactured cured and uncured turkey breast slices, formulated without and with 1.5% potassium lactate and 0.05% sodium diacetate, were inoculated (1–2 log CFU/cm²; one or two replications/three samples each) with *L. monocytogenes*, vacuum-packaged and stored at 4, 7 or 12°C. The growth of *L. monocytogenes* and total bacteria were followed for up to 90 days depending on temperature and product type. The Baranyi model was used to calculate bacterial growth rates and lag times. The temperature dependency of the growth rate of *L. monocytogenes* was modeled by a Ratkowsky-type model.

Results: At 4°C the observed growth rates were 0.04 (cured, with lactate-diacetate), 0.14 (uncured, with lactate-diacetate), 0.21 (cured, no antimicrobials), and 0.35 (uncured, no antimicrobials) log CFU/cm²/

day. Corresponding growth rates at 7°C and 12°C were 0.09, 0.18, 0.36 and 0.56, and 0.25, 0.47, 0.77 and 1.30 log CFU/cm²/day, respectively.

For uncured turkey breast, the growth rate of *L. monocytogenes* was reduced by 60–68% in the presence of lactate-diacetate. Greater reductions (68–81%) were observed on cured turkey breast. The model describes accurately the effect of temperature on the growth rate of the pathogen ($R^2adj = 0.98$). The predictive ability of the developed model was assessed with independent data.

Significance: The developed model may be useful in setting sell-by dates for sliced turkey breast and for optimization of product safety. The model and its findings may also be used in future risk assessments.

P5-29 Predicting the Effects of Storage Temperature on Growth of *Listeria monocytogenes* on Roast Beef Formulated with or without Antimicrobials

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Introduction: Delicatessen meats are considered high risk products for listeriosis and, thus, there is a need for better approaches to enhance their safety. A number of models have been developed for the prediction of growth of *Listeria monocytogenes*. However, these models are often based on data obtained in microbiological media and may overestimate growth in food products.

Purpose: The aim of this study was to model the effects of storage temperature on the growth kinetics of *L. monocytogenes* on commercial Ready-to-Eat roast beef.

Methods: Roast beef slices (5 x 5 cm; 1–2 mm thick), formulated without and with 1.5% potassium lactate and 0.05% sodium diacetate, were inoculated (1–2 log CFU/cm²; two replications/three samples each) with *L. monocytogenes* (10-strain composite), vacuum-packaged and stored at 4, 7 or 12°C. *L. monocytogenes* and total bacteria were enumerated for up to 75 days depending on temperature and product type. Growth rates and lag times were determined by fitting growth curves with the Baranyi model. The obtained growth rates were fitted to a square root model as a function of storage temperature.

Results: For most of the environmental conditions, presence of lactate-diacetate extended lag times and decreased ($P < 0.05$) growth rates of *L. monocytogenes*. For example at 12°C, the observed growth rates were 0.48 (with lactate-diacetate) and 1.31 (no antimicrobials) log CFU/cm²/day. The developed model was validated by comparison with new data generated on roast beef and literature data. The model quantifies accurately the effects of temperature and antimicrobials (1.5% potassium lactate, 0.05% sodium diacetate) on the kinetics of *L. monocytogenes* on vacuum-packaged roast beef.

Significance: The model may be useful in efforts to select storage conditions and safety-based shelf life for control of *L. monocytogenes* on roast beef, may be applicable in development of models for other products, conditions or pathogens, and may be used in new or updated risk assessments.

P5-30 Effect of Thirteen Antimicrobials on Morphology of *Listeria monocytogenes* Cells as Examined with Scanning and Transmission Electron Microscopy

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Introduction: The adaptability of *Listeria monocytogenes* membranes is well-documented as one mechanism that allows the cell to survive and grow at low temperatures and high osmotic stress. Cell membranes act as selective barriers, but may be affected by stresses such antimicrobials.

Purpose: This study examined the effects of acetic (0.4%), citric (0.3%), lactic (0.4%) and octanoic (0.04%) acids; sodium diacetate (0.1%), potassium lactate (0.6%), buffered-sodium-citrate (0.4%), nisin (0.2%), pediocin (0.2%), carvacrol (0.4%), eugenol (0.4%), thymol (0.4%) and monolaurin (0.02%) on *L. monocytogenes* cells via scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Methods: Ten *Listeria monocytogenes* strains were cultured individually in Tryptic Soy Broth with 0.6% yeast extract (TSBYE) (30°C/22 ± 2 h), washed with phosphate-buffered-saline, resuspended in TSBYE plus each antimicrobial and incubated (7°C/72 h). Cultures were washed and then fixed, using a 1% glutaraldehyde in 0.1M NaH_2PO_4 buffer. For SEM analysis, pellets were dried with a graded series of ethanol, sputter-coated and then examined by use of a JSM-6500F field-emission SEM. For TEM analysis, pellets were postfixed in 1% osmium-tetroxide PBS solution, then embedded in epoxy resin. Thin sections of resin were stained with Reynolds lead-citrate and examined by use of a JEOL 2000 EX-II TEM.

Results: Under SEM and TEM, cells exposed to acids and salts appeared elongated and thinner than control cells. Cells exposed to bacteriocins showed cell division but not separation; some dissolution of cell membrane was observed. Under SEM, cells exposed to essential oils and fatty acids/esters had noticeable valleys, and under SEM and TEM some cells appeared stunted. All morphological changes were visible under SEM. Under TEM, surface changes were not visible, but differences such as presence/absence of septum were accentuated. No variation was observed among strains.

Significance: Listeria monocytogenes cells exposed to antimicrobials underwent obvious morphological changes. This information could be valuable when studying antimicrobial mechanisms, as well as in determining potential sequential applications of antimicrobials.

P5-31 Control of *Listeria monocytogenes* on Frankfurters by Dipping in Hops Beta Acids Solutions

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Introduction: Hops beta acids (HBA) are parts of hops flowers used in beer brewing and have shown anti-*listerial* activity in bacteriological broth. The US Department of Agriculture Food Safety and Inspection Service has approved HBA as generally recognized as safe for use to control *Listeria monocytogenes* on Ready-to-Eat (RTE) meat products. However, no

published data are available showing the extent of antiListerial effects of HBA in meat products.

Purpose: This study evaluated the effects of HBA as dipping solutions to control *L. monocytogenes* during storage of frankfurters.

Methods: Frankfurters (two replicates/three samples each) were inoculated (1.9 ± 0.1 log CFU/cm²) with *L. monocytogenes* (10-strain mixture), dipped (2 min, 25 ± 2°C) in HBA solutions (0.03%, 0.06% and 0.1%) or distilled water (DW), then vacuum packaged and stored at 4 or 10°C for up to 90 and 48 days, respectively. Samples were periodically analyzed for pH, water activity (day-0) and microbial survival/growth on Tryptic Soy Agar plus 0.6% yeast extract and PALCAM agar. The data were analyzed with the mixed procedure of SAS.

Results: Dipping in HBA solutions caused immediate reductions ($P < 0.05$) of 1.3–1.6 log CFU/ cm² of *L. monocytogenes* without significant effects ($P > 0.05$) on water activity and pH of frankfurters, whereas DW reduced counts by 1.0 log CFU/ cm². Frankfurters untreated or dipped in DW exhibited pathogen growth after 10 days at 4°C or 4 days at 10°C, respectively, with pathogen counts exceeding 7.0 log CFU/cm² after 50 days (4°C) or 28 days (10°C). Pathogen numbers were completely suppressed ($P < 0.05$) until 30–50 days (4°C) or 20–28 days (10°C) on frankfurters dipped in HBA solutions of different concentrations, and the antilisterial effect increased with increased concentrations (0.03%–0.1%).

Significance: HBA may be considered for use to improve the microbial safety of RTE meat products, provided that future studies on sensory qualities show no adverse effects and that their use is economically feasible.

P5-32 Efficacy of Surface Spray Application of Lauric Arginate Derivative to Control *Listeria monocytogenes* on Roast Beef and Pastrami

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Introduction: Post-processing contamination of the surface and/or purge of vacuum-packaged Ready-to-Eat (RTE) meats with *Listeria monocytogenes* (Lm) poses a significant risk to consumers. Because some RTE meats provide a suitable environment for growth/survival of Lm, a post-processing intervention is required to satisfy regulatory guidelines and enhance safety.

Purpose: The purpose was to evaluate the effectiveness of a surface spray application of antimicrobials to control Lm on pastrami and roast beef.

Methods: Commercial pastrami (ca. 1.5 lbs per piece; 4.0 in. H × 3.5 in. W × 6.0 in. L) and roast beef (ca. 1.0 lbs per piece; 4.5 in. H × 2.0 in. W × 4.5 in. L) were separately surface inoculated with a five-strain mixture of Lm (ca. 7.0 $\log_{10}$ CFU/piece), individually placed in shrink-wrap bags, and surface treated with a 5 or 10% solution of lauric arginate (LAE; CytoGuard® LA) or a 10% solution of LAE containing smoke extracts (LAE-S; CytoGuard® LS 611) via the Sprayed Lethality in Container (SLIC®) technology. Bags were subsequently vacuum-sealed and held at 4°C for 1 h. Surviving Lm were enumerated, using the USDA/ARS package rinse method, by adding 0.1% of peptone water or D/E neutralizing broth to the packages and spread-plating onto MOX agar plates.

Results: Regardless of the concentration and type of antimicrobial, the numbers of Lm on the surface of pastrami or roast beef decreased by ca. 0.85 to 1.8 $\log_{10}$ and 0.5 to 1.7 CFU/package, respectively, within 1 h at 4°C. Slightly fewer cells were recovered (0.3 to 0.7 $\log_{10}$ CFU/package) when 0.1% of peptone water was used as the recovery medium, compared to D/E neutralizing broth, to enumerate Lm from the surface/purge of pastrami and roast beef.

Significance: These data establish the ability of LAE and LAE-S applied by SLIC® to reduce Lm levels on the surface of pastrami and roast beef within 1 h at 4°C.

P5-33 Survival and Growth of *Salmonella enterica* Serovar Weltevreden in Som-Fak, a Thai Low-Salt Fermented Product

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Introduction: Fermentations of raw fish are common in Asia to improve shelf life and safety; however, little is known about survival of pathogens in these products. *Salmonella* Weltevreden is a frequent and increasing cause of human infections in Thailand and has been isolated from fishery products in Asia.

Purpose: The purpose of this study was to investigate if growth of *Salmonella* Weltevreden was inhibited by preservation in som-fak (4% salt, 2% garlic and 2.5% lactic acid).

Methods: Salt, garlic and lactic acid (pH levels as in som-fak) were added to BHI with no glucose added (to imitate fish, which do not contain glucose). *Salmonella* was inoculated at 5×10^3, 5×10^5 or 5×10^7 CFU/ml and growth measured as absorbance at 600 nm. Som-fak with garlic or glucose (or nothing) was prepared and inoculated with either (i) 10^3 CFU *Salmonella* Weltevreden, (ii) 10^6 CFU/g garlic fermenting *Lactobacillus plantarum* or (iii) a combination of the two strains. Samples were vacuum packed, stored at 30°C and analyzed for pH, lactic acid bacteria and *Salmonella* the four following days.

Results: Low inocula of *Salmonella* were inhibited; however, growth occurred with 8% salt, at least 2% garlic or 1.5% lactic acid in the BHI medium when the organism was inoculated at 5×10^7 CFU/ml. The strain did not grow in 4% salt plus 2% garlic, representing the beginning of the fermentation, independently of adding lactic acid (Abs. 600 < 0.1 for 6 days).

The *Salmonella* count increased to > 10^8 CFU/g in som-fak without garlic/glucose within one day; pH decreased only to below 4.5 in som-fak to which garlic or glucose was added independently of addition of starter culture, but this was not sufficient to prevent growth of the inoculated *Salmonella*.

Significance: Salmonella Weltevreden survived well and grew to some extent in som-fak independently of the inhibitory substances, emphasising the importance of preventing contamination.

P5-34 UV Catalysis with Novel TiO_2 Nanofiber Coating and Its Bactericidal Activity against *Escherichia coli* O157:H7

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Introduction: Semi-conductor titanium dioxide has been studied as an effective catalyst for bactericidal activity. Material surface coatings with disinfection properties are of particular interest in food safety and processing applications.

Purpose: The objective of this research was to determine the bactericidal effectiveness of a unique TiO_2 nanofiber coating that is a mixture of nanowire and nanotube with unique conductive properties against *E. coli* O157:H7 in food processing environments.

Methods: Titanium circular plates with a diameter of 25 mm were prepared with TiO_2 nanofiber coating for treatments or without nanofiber coating for controls. The samples were inoculated with suspended *E. coli* O157:H7 at 6 log CFU/ml and exposed to long (365 nm), mid (302 nm) and short (254 nm) wavelengths at a 20 cm distance to 4-watt UV light source for different times at room temperature. UV intensity was measured with an Apogee Ultraviolet Meter. After exposure, samples were rinsed with phosphate buffer solution and were plated on non-selective TSA agar to allow injured cell recovery.

Results: When exposed to UV at 302 nm wavelength, the number of inoculated *E. coli* O157:H7 on the TiO2 coating was reduced by up to 3 logs in 5 min compared to the exposed controls without TiO_2 coating. Photocatalytic oxidation of water with UV light in the presence of oxygen and TiO_2 led to the formation of highly reactive OH-radicals, which effectively inactivated *E. coli* O157:H7. SEM images of *E. coli* O157:H7 on the TiO2 fiber surface exposed to UV showed collapsed cells trapped in the TiO_2 fibers.

Significance: TiO_2 nanofiber coating could prove to be an effective bactericidal surface for food processing equipment. This technology may provide a new tool in the control of cross contamination and recontamination of processed food products.

P5-35 Effect of Antimicrobials on the Growth Kinetics and Morphological Changes of Stressed *Salmonella* Typhimurium

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Introduction: Washing with acetic or lactic acid during poultry processing injures *S.* Typhimurium sublethally and their recovery in poultry at the retail market or home is of great concern for the sake of the consumer. Antimicrobials could serve as additional hurdles to control proliferation of injured pathogens.

Purpose: The antimicrobial activities of å-polylysine and potassium lactate (PL) and sodium diacetate (SDA) mixtures are well documented, but their antimicrobial effects on stressed *S.* Typhimurium have not been investigated.

Methods: Acid stressed *S.* Typhimurium were inoculated on a chicken breast patty containing å–polylysine (0, 1, or 1.5%) or PL/SDA mixtures (0, 1.5, or 3%) and the survival and growth of stressed *S.* Typhimurium at 10, 24 and 35°C were investigated. TEM was used to observe the morphological damage following exposure to å–polylysine and PL/SDA mixtures.

Results: å–polylysine or PL/SDA mixtures added to the chicken breast patty delayed the growth of *S.* Typhimurium. Antimicrobial effects mainly affect lag time in the growth curve, which varied with the strength of stress and storage temperature. The addition of 3% PL/SDA mixture or 1.5% å-polylysine did not inhibit the growth of stressed *S.* Typhimurium at 35°C, but completely inhibited its growth at 10°C and 24°C. On TEM micrographs, most of the cell structures with 2% å–polylysine or 4.5% PL/SDA mixture were collapsed and cytoplasmic materials were released from the cells. On the other hand, both dead and doubling cells were observed with 1% å–polylysine or 1.5% PL/SDA mixture by TEM.

Significance: These results have provided valuable information regarding the effect of the level of å-polylysine or PL/SDA mixture to control stressed *S.* Typhimurium contamination on poultry products. Thus the levels and conditions for antimicrobial usage need to be validated under various conditions.

P5-36 DSC Natural Products as Inhibitors of Growth of *Campylobacter* and *Salmonella* Strains

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Introduction: Increased antibiotic resistance of *Campylobacter* and *Salmonella* is a current problem, and the methods to control microorganisms in foods have not been demonstrated to be totally successful. Thus, alternatives are necessary to control the disease that they cause or the microorganisms in foods. Natural products derived from higher plants are offering a new source of antibacterial agents. The use of plant preparations has been well documented, although only a few species have been screened for biological activity.

Purpose: Here, the biological activity of 28 plant extracts against growth of *C. jejuni*, *C. coli*, *Salmonella* Typhi and *S.* Typhimurium was determined.

Methods: Twenty g of each plant was blended with 100 ml of PBS or ethanol, macerated in a bottle for 24 h and then filtered. The extracts obtained (56 in total) were dried at room temperature and then resuspended in a small volume of PBS or ethanol. A diffusion test on agar was conducted and the minimum bactericidal concentration (MBC) was determined for the most active extracts.

Results: Of the 56 extracts analyzed, three (Mexican lime, plum and sour orange) showed the best antimicrobial activity. The MBC obtained ranged from 1 to 3 mg/ml for strains of *Campylobacter* and 7 to 35 mg/ml for strains of *Salmonella*.

Significance: The active plants analyzed are edible plants that could represent viable alternatives for the control of this microorganism in different circumstances.

P5-37 Antimicrobial Activity of Chitosans and Chitooligosaccharides in Milk and Apple Juice, on *Bacillus cereus* and Spores

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Introduction: Bacillus cereus is a Gram-positive, spore-forming bacterium that is widely distributed in nature. It is a common contaminant in a wide variety of foods, including milk and dairy products, cooked vegetables and meats, fruit juices, and boiled or fried rice, among others.

Purpose: The aim of this work was to evaluate the influence of food interactions on the antibacterial activity of five different molecular weight (MW) chitosans against *B. cereus* (and its spores). Sensory changes of the food matrices, with and without chitosans, were also assessed.

Methods: High, medium and low MW chitosans, as well as chitooligosaccharide (COS) mixtures of < 3 and < 5 kDa, were tested against a fixed inoculum level (10^5 cell/ml) of *B. cereus* and *B. cereus* spores, in a culture medium (used as control) and in two selected food matrices – semi-skimmed milk and apple juice. These parameters were monitored by assessing death and

germination rates. Sensory differences were estimated by a trained panel, using a scale ranging from -3 to 3 (-3 = weaker, 0 = equal, 3 = stronger).

Results: Chitooligosaccharides exhibited a strong interaction with milk, and accordingly lost their efficacy rapidly. After an initial positive inhibitory effect that lasted for 4 to 8 h, said mixtures permitted germination rate and viable counts to increase, approaching the control values. Conversely, initial viable counts were reduced to < 10 CFU/ml by any of the three chitosans tested, by 4 h, whereas a similar result was attained with spores by 72 h (low MW chitosan). Regarding apple juice, *B. cereus* was extremely sensitive to any of the five compounds. Although spore germination was inhibited by all compounds, especially COS, no reduction above 3 log cycles was achieved. Astringency and aftertaste were the most frequently reported organoleptic changes, particularly for high MW chitosan.

Significance: These data suggest that use of this polymer will be limited to food products characterized by low protein contents. The MW plays an important role in the observed antimicrobial activity.

P5-38 DSC Chitosans and Chitooligosaccharides: Antimicrobial Activity on *Bacillus cereus* (and Its Spores)

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Introduction: Bacillus cereus is one of the most frequent food poisoning microorganisms, causing both intoxications and infections. Prevalence of *B. cereus* in processed foods occurs because of its resistance to cooking and pasteurization associated with its spore forming ability.

Purpose: In order to control *B. cereus* growth/sporulation, and hence minimize the aforementioned hazards, several antimicrobials have been tested as food additives. The main goal of this work was thus to assess the effect of different molecular weight (MW) chitosans upon *B. cereus* growth, sporulation and germination.

Methods: Five different MW chitosans/chitooligosaccharides (COS) were tested, at various concentrations, against different inoculum levels of *B. cereus* and of *B. cereus* spores; the ability to prevent sporulation was also assessed. These parameters were monitored by first determining minimum inhibitory/lethal concentrations, and via assessment of death and sporulation rates. Atomic force microscopy (AFM) imaging was used to obtain high resolution images of the effect of the said chitosans on bacterial and spore morphologies.

Results: The antimicrobial effect was strongly dependent on cell stage and on the molecular weight of chitosan: it increased with MW for the vegetative form, and the opposite was true for the spores at 1.0%. COS presented bactericidal action upon *B. cereus* only at 1.0% (w/v), while the higher MW chitosan used (i.e., 615 kDa), required only 0.25% (w/v). In contrast, 0.50% (w/v) of high MW was required to eliminate spores, versus 0.20% (w/v) of COS. AFM imaging suggested that all chitosans tested formed a slimy layer around the spore, while nanoindentation experiments revealed mechanical changes in the bacterial cell wall induced by COS.

Significance: These data suggest that the application of chitosans as food additives or packaging coats appears promising toward preventing contamination by *B. cereus.*

P5-39 Synergistic Effect of UV Irradiation on Chemical Disinfectant Treatments for Reduction of *Bacillus cereus*

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Introduction: Various hurdle techniques are being popularly applied to food industry to reduce bacterial foodborne pathogens.

Purpose: This study investigated the synergistic effect of UV irradiation on chemical disinfectant treatments for reduction of *Bacillus cereus.*

Methods: The reduction of *Bacillus cereus* F4810/72 by various concentrations of disinfectants such as ethanol (10, 30, 40, 50%), hydrogen peroxide (100, 500, 1000, 2000 ppm), sodium hypochlorite (10, 50, 100, 200 ppm) were tested with various exposure doses (6, 96, 216, 360 and 506 mW .s/cm²) of UV lamp (2,537Å).

Results: Three kinds of combined treatments, ethanol/UV, hydrogen peroxide/UV and sodium hypochlorite/UV, exhibited a greater reduction and synergistic benefits. The combined treatments, ethanol/UV, hydrogen peroxide/UV and sodium hypochlorite/UV, exhibited large synergistic effects at levels of 0.4–1.51, 0.18–1.83 and 0.28–1.2 log CFU/ml, respectively. Furthermore, the disinfectant treated *B. cereus* cell walls were defective and were even more severely affected by additional UV treatment.

Significance: If combined treatments of disinfectants and UV irradiation for disinfection are applied to reduce *B. cereus*, the synergistic effect could be expected.

P5-40 Withdrawn

P5-41 Antimicrobial Testing of *Staphylococcus aureus* Strains Isolated from Clinical, Milk and Meat Samples

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Introduction: Staphylococcus aureus, particularly methicillin-resistant (MRSA), is a major cause of hospital acquired and foodborne infections and deaths in the US. It is estimated that hospitalizations due to *S. aureus* increased from 294,570 to 477,927 from 1999 through 2005, and MRSA hospitalizations increased from 127,036 to 278,203. Persistent use of antibiotics is believed to have an impact on antimicrobial drug resistance of many pathogens and is a major problem in the USA.

Purpose: The purpose was to determine the antimicrobial susceptibility profile of 24 *Staphylococcus aureus* strains isolated from clinical, milk and meat samples.

Methods: Twenty-four strains isolated from clinical, meat and milk samples were analyzed for their antibiotic susceptibility against 13 antimicrobial agents (ciprofloxin, doxycycline, enrofloxin, gentamycin, neomycin, norfloxacin, oxfloxacin, oxytetracycline sulphadimindine, sulphamethazole, tetracycline, trimethoprim tylosin and chloramphenicol), using a Spiral Gradient Endpoint Method (SGE) to determine the end concentration (EC), tail end concentration (TEC) and gradient minimum inhibitory concentration (GMIC) based at a breakpoint of 0.5 – 512 µg/ml, based on the recommendations of the Clinical Laboratories Standards Institute.

Results: Six isolates (25%) were resistant to 1 to 3 antibiotics, while 7 (29%) of the strains were resistant to four to six antibiotics and 10 (42%) of the isolates were

resistant to seven or more antibiotics. A large number of these isolates were observed to be highly resistant to neomycin, sulphadimidine (GMIC = & #8805;256µg/ml; EC = & #8805;145 µg/ml; TEC = & #8805; 256 µg/ml), tylosin tartrate (GMIC = 8 µg/ml; EC = 4.16µg/ml; TEC = 4.16 µg/ml) and tetracycline (GMIC = 64 µg/ml; EC = 49 µg/ml; TEC=49 µg/ml) while all (100%) of the isolates were susceptible to enrofloxacin (GMIC = & #8804;1 µg/ml; EC = & #8804;0.7 µg/ml; TEC = & #8804;0.7 µg/ml).

Significance: Our study indicates that there may be an increased trend in the isolation of multiple resistant strains of *Staphylococcus aureus* isolated from clinical and food samples.

P5-42 Assessment of Membrane Integrity Damage of *Clostridium perfringens* and *Vibrio cholerae* by Plant Extracts

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Introduction: Natural compounds have been screened as food additives to control foodborne pathogens. Previous reports of our laboratory showed that methanolic extracts of *Prunus salicin* Lindl., *Acacia farnesiana* L. and *Artemisia ludoviciana* Nutt. have antimicrobial activity against *Vibrio cholerae* and *Clostridium perfringens*. Assessment of viability of bacterial cells is a major requirement in the search of antimicrobial agents. Membrane-impermeative fluorescent probes that can passively diffuse through the cell wall of a bacterium can act as an indicator of cell viability.

Purpose: To evaluate the effect of different plant extracts on viability of two strains of *C. perfringens* and *V. cholerae*, using a two-color fluorescent assay which estimates cell-membrane damage

Methods: Microbes were cultured in Thioglycollate Broth (*C. perfringens*) and Luria Bertani Broth (*V. cholerae*). Methanolic extracts of *P. salicina* (5 mg/ml), *A. farnesiana* (5 mg/ml) and *A. ludoviciana* (10 mg/ml) were added to the cultures and incubated at 37°C overnight. Methanol was used as negative control. Total counts of bacteria with membrane damage were determined with Live/Dead BacLight kit (Molecular Probes, L7012). The staining protocol was carried out following manufacturer instructions. An epifluorescence microscope (Axioskop 40FL) was used to observe the cells. The cells with green fluorescence were enumerated as bacteria with undamaged membrane (i.e., viable).

Results: Counts of green fluorescence of untreated cells varied from 80 to 98% for all strains. On the other hand, green fluorescence counts of negative control cells (methanol treated) varied from 71 to 81%. Indeed the proportion of green fluorescence of cells treated with different extract plants was < 1% for all strains; therefore, all methanolic extracts tested against *C. perfringens* and *V. cholerae* caused membrane damage.

Significance: Here we present the evidence that methanolic extracts of *Prunus salicina* Lindl., *Acacia farnesiana* L. and *Artemisia ludoviciana* Nutt. cause damage of bacterial membranes.

P5-43 *Aspergillus flavus* and *A. niger* Growth Response to Cinnamon Extracts

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Introduction: Antimicrobial activities of extracts from several plants and spices used as flavoring agents in foods have been recognized for many years. However, data on the effect of extracts in combination with other factors on mold growth is scarce. Additionally, there are few models to predict performance when natural preservatives are used in combination with other factors.

Purpose: The objective of this research was to evaluate at selected a_w (0.94, 0.91, or 0.86) and pH 3.5 the effects of different concentrations of cinnamon extracts (alcoholic extract or ethyl acetate extract) on *Aspergillus flavus* and *A. niger* radial growth rate and to model mold response by use of the Weibull model.

Methods: Solid media formulated with every factor combination was inoculated with 10^3 spores/ml and incubated at 27°C for up to 30 days. Periodically during incubation, mold colony diameter was measured to determine radial growth rate. Weibull model was fitted to experimental data, using non-linear regression.

Results: Important differences in mold growth rates were observed among aw, and between type of extract and studied molds. For both molds, decreasing a_w and increasing extract concentration decreased radial growth rate. Increasing antimicrobial concentration at a_w 0.86 had a dramatic effect on *A. flavus* and *A. niger* radial growth rate. Weibull parameters show that *A. flavus* was more sensitive than *A. niger*, and highlight the effects obtained with alcoholic or ethyl acetate extracts.

Significance: The Weibull model adequately described studied mold responses for tested extracts and could be used to evaluate critical antimicrobial concentrations, as well as to model flat or very sharp mold inhibition patterns.

P5-44 Antimicrobial Efficacy of Vanillin and Cinnamic Aldehyde in Peach Puree

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Introduction: Many fruit-based beverages use concentrated purees that are refrigerated or frozen. These processes are associated with high costs and quality reduction. A combined methods approach can be used as an alternative to obtain minimally processed purees.

Purpose: The objective of this work was to test vanillin and cinnamic aldehyde as antimicrobial agents to preserve peach puree.

Methods: Peach purees were obtained (caustic peeling, blanching, and pulping) and stabilized by slight reduction of a_w (0.98, by sucrose addition), pH (3.6, by adding citric acid), and incorporation or not (control) of 500 or 1000 ppm of potassium sorbate, vanillin, or cinnamic aldehyde. Purees were placed in plastic bags and stored at 25°C up to 8 weeks; every seven days, the standard plate, and mold and yeast counts were determined, as well as a_w, pH, moisture content, °Brix and color (Hunter L, a, and b). Sensory tests (9-point hedonic scale) were also performed.

Results: Peach purees formulated without antimicrobials showed microbiological spoilage after the first week of storage; in contrast, those purees containing antimicrobial agents in 500 ppm concentration do not show spoilage (< 10 CFU/g), at least during 2 weeks (vanillin or cinnamic aldehyde) or 4 weeks (Potassium sorbate) at 25°C. Purees formulated with 1000 ppm were microbiologically sound during the 8 weeks of storage, and their moisture content, °Brix, pH, and a_w remained almost constant during storage, with mean values of 71.8%, 26.1, 3.65, and 0.978, respectively. Color evaluation revealed that there were no significant ($P < 0.05$) changes among samples with the studied antimicrobials. In every case, good overall acceptability (initial mean score 7.21) of preserved peach purees,

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even after eight weeks of storage at 25°C (final mean score 7.02), was observed.

Significance: It is feasible to apply the combined methods approach to obtain high quality peach purees with lower costs compared to traditional industrial processes.

P5-45 Reduction of Foodborne Pathogens in a Korean Fermented Fish Product (Jeot-gal) Model System with Natural Antimicrobials

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Introduction: The Korean fermented food industry makes great efforts to produce less acidic and less salty food products in accordance with consumer preferences. To inhibit foodborne pathogens and extend the shelf life of the products, the incorporation of natural antimicrobials may have great advantages in the fermented food industry.

Purpose: Instead of a high-salt storage method, use of natural antimicrobials in a Korean fermented fish product (Jeot-gal) was investigated.

Methods: Fifteen natural antimicrobials (0.01–1%, w/v or v/v) were examined to assess their antimicrobial activity against five foodborne pathogens (*E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, *S.* Typhimurium, and *V. parahaemolyticus*) by MIC or by an in vitro test using a Jeot-gal model system (pH 5.5, 5% NaCl, 10°C).

Results: In the model system, grapefruit seed extract, fermented pollen, lactic acid, and thiamin dilaurylsulfate at the 1% level all showed 5 log CFU/ml reduction in the foodborne pathogens after 6 h, while sulf clam and lysozyme at the 1% level showed 3 log and 1 log CFU/ml reduction, respectively. Grapefruit seed extract and fermented pollen product inactivated the pathogens by 3 log CFU/ml at a 0.1% level.

Significance: If these natural antimicrobials can inhibit pathogens in real Jeot-gal effectively, their use will contribute to the safety of these products.

P5-46 Dietary Exposure to Benzoic Acid from Prepackaged Non-Alcoholic Beverages of Secondary School Students in Hong Kong

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Introduction: Benzoic acid is one of the oldest chemical preservatives and is permitted in a variety of foods and drinks in many countries. Concerns about the overexposure to benzoic acid from beverages, especially in the young population, are increasing. Children are at higher risk as they have high energy intake per kg body weight as well as different dietary patterns and food preferences as compared with adults.

Purpose: The purpose of this study was to evaluate the dietary exposure to benzoic acid from prepackaged non-alcoholic beverages of secondary school students in Hong Kong and assess the associated health risks.

Methods: A total of 211 prepackaged beverages, including (i) soft drink (both diet/light and regular types), (ii) fruit juice, (iii) soy milk, (iv) Chinese tea and (v) coffee/tea were sampled from local markets and their respective benzoic acid concentration was analyzed. The dietary exposure was then estimated by use of local food consumption data and the concentrations of benzoic acid in the beverages tested.

Results: Benzoic acid was detected in 36 (17.1%) beverage samples, including soft drinks and fruit juice, with concentrations ranging from 51 to 580 mg/kg. The average dietary exposure to benzoic acid of secondary school students from prepackaged beverages was 0.31 mg/kg bw/day (ranging from 0.17 to 0.41 mg/kg bw/day in different age and sex groups). High exposure ranged from 0.58 to 1.30 mg/kg bw/day in different age and sex groups.

Significance: Upon normal consumption, the dietary exposures to benzoic acid from prepackaged beverages in both average and high consumers were well below the Acceptable Daily Intake (ADI) (0–5 mg/kg bw), suggesting that secondary school students in Hong Kong were unlikely to experience adverse effects of benzoic acid from prepackaged beverages.

P5-47 DSC Antimicrobial Activity of Edible Plants against Enteropathogenic Bacteria

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Introduction: The food industry is trying to reduce the use of chemical preservatives in foods because of negative consumer perceptions of artificial preservatives and to either completely remove them or to adopt more "natural-green" alternatives. Because historically plants have provided a good source of antimicrobial agents, there is considerable interest in the use of these compounds as food additives, to delay the onset of food spoilage or to prevent the growth of foodborne pathogens.

Purpose: In this study we determined the inhibitory effect of 30 edible plants or seeds (spices, fruits and vegetables) against growth of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Campylobacter jejuni*.

Methods: Twenty g of each plant were blended with 100 ml of methanol or ethanol, macerated in a bottle for 24 h and then filtered. The extracts obtained (54 in total) were dried at room temperature and then resuspended in a small volume of PBS. A diffusion bioassay on agar was conducted and the minimum bactericidal concentration (MBC) was determined for the most active extracts via a microplate method.

Results: Of the 30 plants (60 extracts) evaluated for antimicrobial activity against the bacteria tested using the agar well diffusion bioassay, three extracts (strawberry, Mexican lime and plum) were selected because they showed the greatest activity, and their MBC were determined. The MBC of the extracts ranged from 1.7 to 6 mg/ml for *C. jejuni*, 9.8 to 42.5 mg/ml for *E. coli* O157:H7, 9 to 47 mg/ml for *L. monocytogenes* and 7 to 42.5 mg/ml for strains of *Salmonella*. Fifteen plants did not present any antimicrobial activity against the bacteria tested.

Significance: Here we present three plant extracts that could be alternatives to control growth of these foodborne pathogens. Strawberry, Mexican lime and plum are edible fruits that could be easily added to foods.

P5-48 Estimation of Shelf Life of Ethanol and Peroxide Compounds Sanitizers by the Arrhenius Model

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Introduction: Sterilizing disinfectants are used for disinfection, sterilization, and bleaching of the surrounding environments and water as well as food processing equipment. The level of sterilization is affected by various factors such as density of inorganic compounds, pH, temperature, light, time of exposure to microorganisms, and existence of organic matter. One of the major problems is that sterilizing disinfectants need to be handled within scheduled time as much as possible. Among measures and answers to the problems, an appropriate estimation of substantial stability of major active components of sterilizing disinfectants is the most important thing to be considered. The estimation is critical for in situ application as well as storage management for the product, since it is very unstable outside a certain environment.

Purpose: This study is to establish a safety evaluation model of ethanol and peroxide compounds sanitizers and to predict shelf life of sanitizers according to the storage temperature through an Arrhenius model, by carrying out long term preservation tests and accelerated test of sanitizers with ethanol and peroxide compounds which are currently being sold.

Methods: According to the amount and compounded proportion of the sanitizer ingredients and auxiliary components, 9 targets (4 ethanol types and 5 peroxide compound types) were selected and kept at certain temperatures (20, 25, 30, 35, 40, 45, 50°C); suspension test was conducted every month and the actual results were analyzed and compared with the shelf life which was predicted according to the interrelationship between temperature and stress, by using the Arrhenius model.

Results: According to the results, P-6, a peroxide type product, showed the highest effect against *E. coli,* with 358 days, and all the ethanol type sanitizers did not demonstrate any reduced effects during the study at any given temperature, which showed great stability. In addition, predicted results using the Arrhenius model and the actual results for peroxide type sanitizers were very similar.

Significance: Subsequently, peroxide type sanitizers show a disproportionate relationship between temperature and shelf life, so users of peroxide sanitizers should pay close attention to the temperature in storing them.

P5-49 Evaluation of Antimicrobial Properties of Lemongrass [*Cymbopogon citratus* (*C. Nees*) Stapf (Poaceae)]

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Introduction: The increasing resistance to antibiotics of bacterial pathogens has raised concerns in the scientific community. Medicinal herbs, such as lemongrass, have the potential to serve as alternative methods for control of these foodborne pathogens.

Purpose: This study evaluates the antimicrobial properties of lemongrass extracts (derived using different solvents and drying methods) against foodborne pathogens.

Methods: Lemongrass leaves and stem samples were dried (freeze-dried or oven-dried) and extracted with solvents (water, ethanol, acetone and hexane). An antimicrobial assay was performed against *Staphylococcus aureus* (ATCC 29247, 12600U, and 35548), *Escherichia* coli O157:H7 (204P, 301C and 505B), and *Salmonella* serotypes Enteritidis, Typhimurium, Mission and Thompson (ATCC 8391) strains, using the agar disc diffusion method.

Results: Among the bacterial strains tested, *Staphylococcus aureus* strains were sensitive to the hexane and acetone extracts from the lemongrass stems. None of the leaf samples were shown to have any antimicrobial activity, no matter which solvents or drying methods were used. The inhibitory zone ranged from 6.5 mm to 21 mm. Oven-drying methods yielded significantly higher ($P < 0.0001$) antimicrobial activity than freeze-drying methods, and in the oven-dried samples, hexane extracts yielded significantly higher ($P < 0.0001$) activity than acetone extracts. Seasonal variations were observed, with plants from late in the season showing greater activity ($P < 0.0001$) than plants from earlier in the season.

Significance: Hexane and acetone extracts of oven-dried lemongrass stems exhibit antimicrobial activity against *Staphylococcus aureus.* Lemongrass stem extract has potential for use in controlling *Staphylococcus aureus.*

P5-50 Antibacterial Activities of Metal Nanoparticle Catalysts

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Introduction: In macrometallic form, metals such as copper and silver demonstrate broad-spectrum antimicrobial activities. In nanoparticulate form, these metals and their alloys may not only retain these properties, but also be amenable to incorporation into antimicrobial fabrics or biofilm-resistant surfaces.

Purpose: The purpose of this study was to evaluate the antibacterial activities of commercially available metal nanoparticle catalysts. Particles examined included copper, silver and a novel copper-silver alloy.

Methods: Metal nanoparticle catalysts (Cu, Ag, Cu-Ag alloy) were sourced from QuantumSphere, Inc. (Santa Ana, CA). Particles ranged in size from 10 to 100 nm and were suspended in a non-polar solvent (dimethyl formamide) for delivery into aqueous media. Particles were tested against *Escherichia coli* ATCC 25922 with broth microdilution or time course plating and were examined in both a rich medium (Mueller-Hinton broth) and a simple buffer system (phosphate buffered saline). Cell-particle interactions were examined via scanning electron microscopy, and nanoparticle-based effects on cellular respiration were measured using the fluorescent respiratory substrate 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), combined with fluorescence microscopy and flow cytometry.

Results: In the broth system, we found that the Cu-Ag alloy particles were most antimicrobial, with a minimum inhibitory concentration of 250 ppm (vs. 500 ppm for Ag and 1,000 ppm for Cu). All particles showed higher activities in the buffer system, as determined by plating. In this system, however, the Cu particles were the most effective. Experiments using coupling flow cytometry with CTC labeling showed that the Cu-Ag alloy (250 ppm, 90 min) almost completely inhibited cellular respiration, suggesting a potential mechanism of action for these particles.

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Significance: Our results suggest that these particles may be promising antimicrobial materials. It may be possible to incorporate these particles into fabrics, rubber or plastics to yield antimicrobial articles of clothing or biofilm-resistant materials of value to the food processing industry.

P5-51 DSC Polyionic Compounds Enhance the Antimicrobial Activities of Plant Essential Oils

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Introduction: Plant extracts have been used for millennia for the treatment of disease, and much recent interest has focused on the antimicrobial activities of plant essential oils (EOs). Although these oils are active against common foodborne pathogens, their effective use as topical, environmental or food antimicrobials will require EO-based formulations with enhanced antimicrobial activities.

Purpose: The purpose of this study was to evaluate two polyionic compounds for their abilities to enhance the antimicrobial activities of select EOs against *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Methods: EOs tested included cinnamon, clove and oregano. Polyionic compounds tested were polyethyleneimine (PEI, polycationic) and sodium poly-phosphate (SPP, polyanionic). EOs were assayed for activity both alone and in the presence of polyionic compounds against *E. coli* O157:H7 ATCC 35150 and *L. monocytogenes* F6854. Test methods included disk diffusion and broth microdilution, using Mueller-Hinton agar or broth. For disk diffusion tests, cells (10^6 CFU/ml) (and 50 µg/ml PEI or 1% SPP, where appropriate) were seeded into molten, tempered agar and overlaid onto base agar plates. Sterile disks saturated with EOs were placed on hardened overlays, plates incubated at 30°C for 18–24 h, and zones of inhibition (ZOI) measured. Broth microdilution assays were performed at 30°C for 18–24 h using an automated turbidimeter.

Results: Both PEI and SPP were effective at enhancing the antimicrobial activities of EOs against the strains tested here. For example, co-treatment of *E. coli* O157:H7 with PEI or SPP and cinnamon oil increased disk diffusion ZOI from 17 mm (control) to 33 mm. Addition of PEI to oregano oil reduced the broth dilution MIC for *Listeria monocytogenes* from 0.25% to 0.0156%.

Significance: Collectively, our data demonstrate that both PEI and SPP can enhance the activities of select EOs. This may lead to the development of more effective EO-based antimicrobial treatments for topical, environmental or food use.

P5-52 The In Vitro Activity of Antibacterial Foam Handwashes Can Vary Dramatically and is Not Strictly Dependent upon the Active Ingredient or Active Concentration

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Introduction: A growing trend among handwashes is the "foam" format, which eliminates the time required to generate lather and enables the user to focus on proper handwashing technique. However, little information has been published to date regarding the antibacterial activity of these products.

Purpose: The purpose was to evaluate the bactericidal activity of five commercial antibacterial foam handwash products against bacteria particularly important to the food industry.

Methods: Four commercially available antibacterial foam handwashes with chloroxylenol or triclosan active ingredients were chosen based on prevalence in the food industry and active ingredient. A fifth foam handwash sold to the healthcare industry was also evaluated. An in-vitro time-kill method was used to evaluate product efficacy in triplicate at 15 and 30 s when challenged with 29 bacterial species and strains. An ANOVA with multiple comparisons was conducted to identify differences in the antibacterial efficacy (α = 0.05).

Results: PX1 (0.5% chloroxylenol) and TC1 (0.3% triclosan) achieved at least a 4 $\log_{10}$ (99.99%) reduction of 22 of the 29 strains after 15 s, were statistically equivalent, and outperformed all other products overall ($P < 0.05$). PX2 (0.5% chloroxylenol) and TC3 (0.45% triclosan) achieved at least a 4 $\log_{10}$ reduction of 18 and 11 strains in 15 s respectively, were statistically equivalent, and were statistically superior to TC2 (0.3% triclosan), which achieved at least a 4 $\log_{10}$ reduction of only 6 strains after 15 s.

Significance: These results demonstrate that either triclosan or chloroxylenol can be used to formulate a broad spectrum antibacterial foam handwash. However, the concentration of chloroxylenol or triclosan in foam handwashes cannot be used as an accurate predictor of overall in vitro antibacterial performance; efficacy against one or two bacteria does not predict broad spectrum activity. Therefore, selection of an antibacterial foaming handwash should be based on data rather than the active ingredient choice or concentration.

P5-53 DSC Screening and Usage of Bacteriocin-Like Inhibitory Substance from Senegalese Traditional Foods Lactic Acid Bacteria as Fish Preservative

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Introduction: Food biopreservation using lactic acid bacteria (LAB) bacteriocins (antimicrobial peptides) as sanitizers can be performed with soft equipment, so that it is easily reproducible in developing countries.

Purpose: A screening of bacteriocin-like inhibitory substances (bac+) from Senegalese traditional foods LAB strains was undertaken. Bactericidal culture supernatant of the main screened strain supplemented with sodium chloride was used as a preservative on sumpat grunt fish, predominant in artisanal fishery landings in Senegal.

Methods: A total of 220 LAB strains randomly selected from 32 samples of local food products (dairy, cereal, seafood) were tested for bacteriocin production by a well diffusion technique. Bac+ strains were characterized by API 50CH tests and 16S rDNA sequencing, as well as a screening of bacteriocin genes by PCR methods. For preservative assays, 100 ml of culture supernatants of the main screened bacteriocin producer (CWBI-B1410) supplemented with sodium chloride was added to 100 g of fillets from fish purchased at a Senegalese local market, which were stored at 10°C. Total viable microbial counts of these fillets were compared to those of fillets treated with a non-bacteriocin producer (LMG 6890), using a 10^6 CFU/g microbial counts level as the end of the shelf life.

Results: Twelve bac+ strains were detected. Eleven were identified as *Lactococcus lactis* subsp. *lactis* strains containing the nisin gene and one *Enterococcus faecium* with the enterocin B gene. Shelf life of raw fish fillets with added 0.08, 0.12, and 0.14 g/ml NaCl non-bactericidal LMG 6890 culture supernatants were determined to be 3, 6 and 7 days, respectively. The use of 0.08, 0.12, and 0.14 g/ml NaCl bactericidal *Lactococcus lactis* CWBI-B1410 culture supernatants as sanitizers delayed the increase of bacteria number in fillets, resulting in a shelf-life extension of 4, 7, and 12 days, respectively.

Significance: These data suggest that this soft technology can be a suitable strategy to improve storage and bacterial quality of tropical fish products.

P5-54 DSC The Bacteriocin-Producing *Lactococcus lactis* CWBI-B1410 Supplemented with Glucose is a Protective Culture to Improve the Bacterial Spoilage Control of Traditional Fish Products Fermented in Senegal

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Introduction: Lactic acid bacteria (LAB) are used as natural and selected starters in food fermentations. However, seafood fermentation is difficult because of their low content of carbohydrate, which is the carbon source for LAB.

Purpose: The bacteriocin (antimicrobial peptide) producing *Lactococcus lactis* CWBI-B1410 strain isolated from Senegalese traditional fermented millet was tested as a protective culture on sumpat grunt *(Pomadasys jubelini)* and giant African threadfin *(Polydactylus quadrifilis)* fishes fermented in Senegal.

Methods: Fish samples purchased at a local market were filleted at the laboratory. Fillets of 100 g supplemented with 1% (wt/wt) glucose were inoculated with 10^7 CFU/g CWBI-B1410 living cultures, and incubated for 24 h at 30°C for fermentation. Spoilage bacterial counts on selective media [Hektoen (*Enterobacteriaceae* and certain *Bacillus* sp. strains isolation) and on Rose Gal Bicig (*Escherichia coli* strains isolation)] and pH were determined at regular intervals. These levels were compared to those of fillets not treated (traditional processing) as well as fillets inoculated with CWBI-B1410 without glucose supplementation.

Results: Bacterial counts in raw fillets assessed at [10^4 (Hektoen) and 10^{1-2} (Rose Gal Bicig) CFU/g] were determined respectively at [10^4–10^5 CFU/g and non detectable] in treated *P. jubelini*, and [10^6–10^7 and 10^2 CFU/g] in treated *P. quadrifilis* fillets at the end of the fermentation. Bacteriocin activity (1280 AU/ml in fish juice) and pH decrease (4.5) were observed in these fillets. Bacterial counts of untreated fillets as those inoculated with CWBI B1410 without glucose addition reached (Hektoen) and 10^3–10^4 (Rose Gal Bicig) CFU/g after 9 h of incubation, with predominance of putrescent Proteus sp strains. Any bacteriocin activity and pH decrease were observed in these fillets.

Significance: These data suggest that CWBI-B1410 strain with carbohydrate supplementation is a protective culture for enhancing bacterial quality of fish products fermented in Senegal.

P5-55 Assessing the Efficacy of Home-Style Cooking Methods on Reducing Tetracycline Resistant Bacteria in Shrimp Samples

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Introduction: The wide distribution of antibiotic resistant (ART) commensal bacteria in the food chain has become a public health concern. These bacteria may serve as a significant avenue transmitting antibiotic resistance (AR) genes to human pathogens. While it is going to be a long-term and expensive effort to clean up the resistance gene pool in the environment, minimizing the ART bacteria through proper food processing might be an effective way to control the transmission of AR to humans through the food chain.

Purpose: The aim of this study was to evaluate the efficacy of home-style cooking methods on minimizing the load of tetracycline resistant (Tetr) bacteria in shrimp samples.

Methods: Shrimp samples were purchased from grocery stores and treated by boiling, stir frying or steaming, followed by storage at refrigeration temperature for up to 48 h. ART bacteria were recovered every 12 h on BHI-Tet plates and the presence of three tetr genes (tetM, tetS, tetL) was examined. Isolated Tetr bacteria were identified by partial 16S rRNA gene sequence analysis.

Results: Our studies showed that all tested cooking methods significantly reduced the population of Tetr bacteria in shrimp samples. However, although no ART bacteria were detected directly on BHI-Tet plate after the shrimp were boiled for 15 mins, ART bacteria were recovered after overnight incubation in BHI broth prior to plating on BHI-Tet plate, indicating that some ART bacteria were injured but not killed under the processing condition. Identified AR gene carriers from cooked shrimp samples included *Carnobacterium* sp. (tetS), *Enterococcus* sp. (tetM, tetL) and *Lactococcus* sp. (tetM).

Significance: Our results suggested that conventional household cooking methods can effectively minimize the population of ART bacteria in shrimp samples. Proper food processing can be an effective option to control the dissemination of AR to humans through the food chain.

P5-56 Chemical Characterization and Histamine-Forming Bacteria in Salted Mullet Roe Products

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Introduction: Grey mullet (*Mugil cephalus*) is one of the most widely distributed food fish in the world. Mullet roe is especially popular in Taiwan and Japan, where it is processed into a dried and salted product. The traditional processing of mullet roe involves salting, desalting and sun-drying, and the final product is a yellow-brown color with about 4% salt content and 20–30% moisture content.

Purpose: The purpose of this research was to test salted mullet roe products sold in the retail markets in Taiwan to better understand their bacterial and chemical quality.

Methods: Sixteen salted mullet roe products sold in retail markets in Taiwan were purchased and tested to determine the contents of aerobic plate count, total

coliforms, *E. coli*, total volatile basic nitrogen (TVBN), and biogenic amines (including histamine). The histamine-forming bacteria were isolated from Niven's medium and identified by 16S rDNA sequencing with PCR amplification.

Results: The pH, salt content, water content, total volatile basic nitrogen (TVBN) and aerobic plate count (APC) in all samples ranged from 5.4 to 5.8, 5.1 to 7.2%, 15.4 to 27.3%, 32.0 to 69.6 mg/100 g and <1.0 to 7.1 log CFU/g, respectively, and none of these samples were positive for total coliforms and *E. coli*. The average content of each of the nine biogenic amines in all samples was less than 4 mg/100 g. The two *Staphylococcus carnosus* strains isolated from this test sample were found to produce a low level of histamine in culture broth.

Significance: This study to determine the safety of 16 salted mullet roe products sold in Taiwan showed that they had satisfactory bacterial quality with no total coliforms and *E. coli*. However, the TVBN content in all samples exceeded the 30 mg/100 g decomposition limit.

P5-57 DSC The Effect of Storage Temperature on the Growth and Survival of Total and Pathogenic *Vibrio parahaemolyticus* in Gulf Coast Shell Stock Oysters

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Introduction: Information is limited on *Vibrio parahaemolyticus* (Vp) growth and survival in oysters under various storage conditions.

Purpose: This study evaluated the effect of storage temperature on the growth and survival of total and pathogenic Vp in post-harvest shell-stock oysters.

Methods: Oysters harvested from Alabama waters in Spring 2007 were stored at 5, 10, 20, 25, and 30°C for selected time intervals. At each time interval, two replicates of six oysters each were analyzed for total Vp levels by direct plating/DNA probe for the species specific thermolabile hemolysin (tlh) gene. Pathogenic Vp levels were determined by MPN-qPCR analysis targeting the thermostable direct hemolysin (tdh) and thermostable-related hemolysin (trh) genes. The Baranyi D and linear models were fitted to the Vp growth and survival data to estimate the maximum growth rate (GR).

Results: GR estimates of total Vp at 5, 10, 15, 20, 25, and 30°C were 0.0005, 0.015, 0.061, 0.047, 0.12 and 0.17 log CFU/h, respectively. Assuming a linear model for the initial growth phase, the best estimates of GR of tdh- and trh- positive Vp at 5, 10, 15, 25, and 30°C were 0.006, < 0.001, 0.16, 0.27, 0.15, and 0.024, 0.006, 0.25, 0.21 and 0.16 log MPN/h, respectively. The GR of total, tdh- and trh- posltive Vp were similar at 30°C.

However, the GR of pathogenic Vp were substantially greater at lower temperatures than those observed for total Vp.

Significance: The growth rates of total Vp were in agreement with those used in the FDA Vp quantitative risk assessment, but pathogenic Vp may multiply more rapidly at lower temperatures (10–25°C) than previously assumed. Analysis of similar data sets from the summer and fall of 2007 is under way to confirm these findings, which may be of value for refining existing risk assessment models and developing Vp control plans.

P5-58 Predictive Model for the Growth and Survival of *Vibrio vulnificus* in Gulf Coast Shellstock Oysters

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Introduction: Vibrio vulnificus (Vv), an opportunistic human pathogen found in the estuarine environment, is the leading cause of reported human death in the US associated with the consumption of seafood. Vv can cause septicemia in susceptible individuals and most cases are linked to consumption of raw oysters. Post-harvest growth of Vv in oysters can greatly increase risk, but limited information is available on growth and survival of Vv in post-harvest oysters. The reliability of predictive models for the growth and survival of Vv in shellstock oysters harvested from Gulf Coast would benefit from more comprehensive information.

Purpose: The objective of this study was to address this existing data gap.

Methods: Oysters were collected seasonally (spring, summer and fall 2007) from the GC and stored at 5, 10, 15, 20, 25, and 30°C. Two samples of six oysters each were analyzed at selected time intervals, using a most probable number (MPN) procedure. The Baranyi D-model was fit to the resulting Vv growth and survival data to estimate the parameters of lag phase duration (LPD) and growth/inactivation rate (GR).

Results: Vv was found to be inactivated at 5 and 10°C with a rate of -0.0144 and -0.0064 log MPN/h, respectively. Except for the spring 10°C samples, no LPD or GR 'shoulder' was observed. The maximum GR (0.059 log MPN/h) was observed at 30°C. At 15, 20 and 25°C, the GRs were 0.0131, 0.0409, and 0.0507 log MPN/h, respectively. The bias and accuracy factors for a square root secondary growth model were 0.9998 and 1.045, respectively. GRs were similar over the three seasons but lower than the assumptions made in the FAO/WHO Vv Quantitative risk assessment.

Significance: The results of this study will assist risk managers and the seafood industry in designing and implementing food safety plans to minimize the risk from Vv in seafood consumers.

P5-59 DSC Enumeration and Molecular Characterization of *Vibrio parahaemolyticus* in and Isolated from Louisiana Gulf Coast Oysters

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Introduction: Enumeration of *Vibrio parahaemolyticus* is complicated because only a small proportion of the population is believed to be capable of causing disease. Previous studies on the levels of *V. parahaemolyticus* in molluscan shellfish do not discriminate between pathogenic and non-pathogenic strains.

Purpose: The purpose of this study was to enumerate the pathogenic portion of the *V. parahaemolyticus* population present in Louisiana Gulf Coast oysters. Pathogenic strain isolates were genotyped by pulsed field gel electrophoresis (PFGE) and direct genome restriction enzyme analysis (DGREA).

Methods: Oysters were harvested from 2–3 sites seasonally over a two year period (2006–2007) from the Louisiana Gulf Coast. Pathogenic *V. parahaemolyticus*

were enumerated with a multiplexed MPN-PCR targeting the thermostable direct hemolysin (tdh) and tdh-related (trh) genes; the multiplex reaction included the species-specific thermolabile hemolysin (tlh) to evaluate total *V. parahaemolyticus* numbers. Pathogenic isolates were obtained from positive MPN enrichments after identification by colony lift hybridization. PFGE was done by use of NotI and SfiI restriction enzymes; DGREA was performed with NaeI. Typing data was analyzed with Bionumerics software.

Results: The numbers of pathogenic *V. parahaemolyticus* correlated with water temperature such that higher MPN values were observed during spring/summer. The highest MPN (based on the tdh gene) for a single site was 779 MPN/100 g oyster meat. In all but two seasons, (winter/spring 2007), the total *V. parahaemolyticus* counts (based on the tlh gene) exceeded the limit of detection of 1100 MPN/100 g oyster meat. In these two seasons, the percent of tdh positive strains were 6.3 and 12.2, respectively. Sixty-eight isolates containing tdh and/or trh genes were genotyped. Many isolates appeared to be identical, but there were instances where related strains clustered by geographical location.

Significance: This data shows that the prevalence of pathogenic *V. parahaemolyticus* strains (tdh and trh-positive) may be higher than previously believed for Louisiana Gulf Coast oysters.

P5-60 Reduction of *Vibrio parahaemolyticus* in Pacific Oysters during Long-Term Frozen Storage

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Introduction: Frozen storage is commonly used to prevent bacterial growth in food. Certain oyster producers have utilized the technology to deliver high quality frozen oysters for raw consumption by consumers. However, no studies have reported the effects of frozen storage on reducing *V. parahaemolyticus* in Pacific oysters.

Purpose: This study investigated the efficacy of long-term frozen storage (-10, -23 and 30°C) on reducing *V. parahaemolyticus* in half-shell Pacific oysters.

Methods: Raw Pacific oysters were inoculated with a five-strain cocktail of *V. parahaemolyticus* to a level of approximately 3.5×10^5 MPN/g. Inoculated oysters were sent through a liquid nitrogen gas tunnel (-95.5°C /-140°F, 12 min) for quick-freezing. Frozen oysters were glazed with tap water from a spray faucet and stored in cardboard boxes at -10, -23 and -30°C. Populations of *V. parahaemolyticus* in oysters were determined (5 oysters each) before and after the freezing process and once a month for up to 4 months, using the FDA's 3-tube MPN method.

Results: The ultra-low freezing process had no effects on inactivating *V. parahaemolyticus* in oysters. Populations of *V. parahaemolyticus* in inoculated oysters remained almost unchanged after the quick-freezing process. However, subsequent storage of frozen oysters at -10, -23, and -30°C resulted in considerable decreases in *V. parahaemolyticus* counts. The populations of *V. parahaemolyticus* in oysters declined by 2.45-, 1.71-, and 1.45-log MPN/g during one month of storage at -10, -23, and -30°C, respectively. The decreases of *V. parahaemolyticus* populations in frozen oysters increased to 3.82 (-10°C), 3.14 (-23°C), and 2.28 (-30°C) log MPN/g at four months of storage.

Significance: Frozen storage of raw oysters at -10 or -23°C for four months could be used as a post-harvest processing method to achieve greater than 3-log (MPN/g) reductions of *V. parahaemolyticus* in oysters.

P5-61 Irradiation D-10 Values and UV Destruction of Finfish Isolates of *Listeria monocytogenes*

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Introduction: Recently, *Listeria monocytogenes* has been isolated from both raw and smoked finfish. There is little data on this pathogen's resistance to either gamma or ultraviolet radiation.

Purpose: This study determined the radiation D-10 values (gamma and ultraviolet radiation) of four finfish *L. monocytogenes* isolates along with *L. monocytogenes* Scott A and the avirulent *L. welshimeri*.

Methods: Each isolate was inoculated on raw refrigerated and frozen finfish and irradiated at dose levels ranging from 0.4 to 2.4 kGy at 4°C or -10°C. The ultraviolet radiation resistance of each isolate was determined on agar, using a combination of 185 and 254 nm UVC light source measuring 0.8 mW/cm^2.

Results: One isolate, NFPA7459, had a significantly higher ($P > 0.05$) irradiation D-10 value. The average D-10 radiation value (n = 80) obtained was 0.62 ± 0.09 kGy, which is typical for those reported for *Listeria*. The inoculation level was 8.9 log CFU/ml for each isolate and a 7 log reduction resulted after a UV radiation dose of 4 mW/s/cm^2 (4 Joules/s/cm^2).

Significance: The data obtained indicated that a multi-isolate cocktail can be used for future inactivation studies of *L. monocytogenes* on finfish.

P5-62 DSC Potential Application of Antimicrobials to Control *Listeria monocytogenes* in Vacuum-Packaged Cold-Smoked Salmon (CSS) Pâté and Fillets

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Introduction: Listeria monocytogenes (LM) contamination of Ready-to-eat seafood products has become a significant concern. In the face of numerous incidents of LM contamination and an increasing desire for minimally processed foods, interest by the food industry in using natural antimicrobials to control LM remains high.

Purpose: The first objective was to determine the minimum inhibitory concentrations (MICs) of nisin, sodium lactate (SL), sodium diacetate (SD), sodium benzoate (SB), and potassium sorbate (pS) against LM. The antilisterial efficacy of binary antimicrobial combinations in vitro and on smoked salmon pâté and fillets were subsequently evaluated.

Methods: Cultures of twelve strains of LM were separately diluted to ~ 10^5 CFU/ml in sterile TSBYE (pH 6.8) and 50 ul dispensed into wells of a 96-well plate. Fifty ul antimicrobial aliquots serially diluted in 0.02 M acetic acid was added and samples were incubated overnight at 35°C. Binary mixtures of antimicrobials were then tested in TSBYE inoculated with a cocktail of two antimicrobial-resistant strains (~10^5 CFU/ml) and incubated overnight at 35°C. Selected effective combinations were then verified in real foods by direct addition into smoked salmon pâté formulation or surface application on salmon fillets pre-inoculated with LM to a density of ~ 10^3 CFU/g and stored at 4°C for 3 and 6 weeks, respectively.

Results: MICs for nisin, SL, SD, SB and PS were strain-dependent and ranged from 0.00048–0.00190%,

Poster

4.60–5.60%, 0.11–0.22%, 0.25–0.50% and 0.38–0.75%, respectively. The two most effective antimicrobial formulations on pâté (0.25% SD and 2.4% SL/0.125% SD) significantly ($P < 0.05$) suppressed LM by 3.5 $\log_{10}$CFU/g relative to the control. Application of 2.4% SL/0.125% SD, 0.25% SD or 0.00125% Nisin/0.15%PS on fillets represented the most effective treatments over three weeks' storage, significantly ($P < 0.05$) reducing the counts by 3.4 $\log_{10}$CFU/g.

Significance: These antimicrobial treatments may feasibly assist the smoked salmon industry in its efforts to control LM.

P5-63 DSC Isolation and Characterization of *Listeria monocytogenes* from Blue Crab Meat (*Callinectus sapidus*) and Blue Crab Processing Plants

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Introduction: Listeria monocytogenes is a Gram positive, intracellular foodborne pathogen that causes a severe disease called listeriosis in high risk groups. However, there is limited information about the prevalence and sources of *L. monocytogenes* in blue crab and blue crab processing plants in Maryland.

Purpose: The purpose of this study was to address this data gap.

Methods: For this study, samples were collected from seven processing plants in Maryland from May through November 2006. A total of 272 raw crabs, 344 finished product, and 344 environmental sponge samples were analyzed by the US Food and Drug Administration Bacteriological Analytical Method. Presumptive *L. monocytogenes* was confirmed by the BAX Polymerase Chain Reaction and API *Listeria* Kit. To track the origin and spread of *L. monocytogenes*, one isolate from each positive sample was subtyped by ribotyping and pulsed-field gel electrophoresis (PFGE).

Results: Listeria monocytogenes was isolated from 6.3% of raw crabs, 0.3% of finished products and 3.5% of environmental samples. Among the environmental sites, the most contaminated were raw crab coolers (11.6%) and receiving docks (6.8%). Automated EcoRI ribotyping differentiated seven ribotypes among the 30 *L. monocytogenes* isolates. For each of the four plants with *L. monocytogenes* positive environmental samples, one or two ribotypes appeared to persist in the plant environment during the study period. In one plant, a specific ribotype that persisted in raw crab and an environmental site might have been responsible for contamination in one finished product. Twenty-six different pulsotypes were recovered from 30 *L. monocytogenes* isolates. Fifteen and 11 pulsotypes were recovered from raw crabs and environmental samples, respectively. Indistinguishable pulsotypes were observed both in raw crabs and in several environmental sites.

Significance: These results indicate that raw crabs are an important initial source of *L. monocytogenes* contamination in blue crab processing plants.

P5-64 Efficacy of FD&C Red No. 3 and Ultra-High Pressure Combination Treatment against Foodborne Pathogens in Food Systems

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Introduction: The combination of FD&C Red No. 3 and ultra-high pressure (UHP) synergistically inactivates processing-resistant strains of spoilage and pathogenic bacteria. Often, emerging preservation methods are effective against target organisms in simple buffer systems but ineffective against these organisms in complex systems, including food.

Purpose: The objective of this study was to determine the efficacy of UHP-Red 3 against processing-resistant *Listeria monocytogenes* and *Escherichia coli* O157:H7 in two food systems.

Methods: L. monocytogenes OSY–328 and *E. coli* O157:H7 EDL 933 were inoculated into sterile carrot juice and sterile turkey meat product containing 0–100 ppm Red 3. Inoculated food products were subjected to UHP (500 MPa, 1 min) and bacteria were recovered on Trypticase Soy Agar following incubation at 37°C for 48 h.

Results: UHP in combination with 10, 50, and 100 ppm Red 3 resulted in synergistic inactivation of *L. monocytogenes* in carrot juice. UHP alone resulted in 2.6 log reduction of *L. monocytogenes*, whereas UHP-Red 3 (100 ppm) inactivated 6.7 log. UHP-Red 3 (100 ppm) synergistically inactivated *E. coli* in carrot juice, with reduction of 4.8 log. UHP treatment was ineffective against *L. monocytogenes* in turkey meat product; likewise, combination treatment did not produce significant inactivation. UHP treatment reduced *E. coli* in turkey meat product by 3.9 log; however, combination treatment did produce significant increases in inactivation, regardless of Red No. 3 concentration.

Significance: Food components significantly impact the efficacy of UHP-Red 3 combination treatments. These combinations are effective in carrot juice, but ineffective in a meat system. Further studies are needed to identify specific components that protect bacteria from inactivation by both UHP and Red 3 treatments.

P5-65 DSC The Survival of *Escherichia coli* in Rainbow Trout Byproducts (*Oncorhynchus mykiss*) Processed Using Isoelectric Solubilization / Precipitation

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Introduction: Protein and lipids typically wasted by the disposal of fish processing byproducts may be recovered using isoelectric solubilization/precipitation. The protein is solubilized at an extreme pH and then recovered by precipitation and centrifugation. Microbial survival throughout this process has not yet been determined.

Purpose: The purpose of this study was to determine the survival of *Escherichia coli* following exposure to an extreme pH shift during the protein and lipid recovery process.

Methods: Fresh rainbow trout byproducts (heads and frames) were inoculated with *E. coli* ATCC 25922, homogenized, brought to the target pH of 3.0 by the addition of concentrated hydrochloric acid, held at 4°C for 10 min, and separated into their constituents (lipid, water, soluble protein, and insoluble components) via centrifugation. The lipid and insoluble components (bones, skin, insoluble protein, etc.) were removed. A second pH shift to 5.5 was used to separate the soluble protein and water fractions. Each constituent was analyzed for bacterial content, using selective violet red bile agar and non-selective Tryptic Soy Agar, with the sum of the surviving *E. coli* in these parts being compared to the initial inoculum.

Results: Compared to the initial inoculum level, there was no significant ($P > 0.05$) reduction of total *E. coli* when the pH was shifted to pH 3.0. Most of the survivors were located in the insoluble components fraction and the fewest in the water fraction. There was a 1.3 log and 2.2 log reduction of *E. coli* in the recovered protein and lipid fractions, respectively.

Significance: Protein and lipid recovery by isoelectric solubilization/precipitation using a pH of 3.0 is not sufficient to significantly reduce *E. coli* ATCC 25922. Reducing the pH further and increasing the exposure time may result in significant reductions of bacteria.

P5-66 Effect of Pressurization Rate and Food Matrix on Spore Inactivation by Pressure-Assisted Thermal Processing

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Introduction: Pressure-assisted thermal processing (PATP) provides an opportunity to process superior quality shelf-stable low-acid foods. During PATP, elevated pressure (500–700 MPa) in combination with heat (90–121°C) is used to inactivate harmful bacterial spores.

Purpose: The objectives of this study were to investigate the effect of pressurization rate and food matrix on the inactivation of *Bacillus amyloliquefaciens* and *B. stearothermophilus* spores during PATP treatments.

Methods: Spore suspensions inoculated in egg patty mince (PH 6.28, a_w 0.99; ~1.6×10^8 CFU/g) and tomato paste (PH 4.14, a_w 0.96; ~1.1×10^8 CFU/g) were processed at 600 MPa and 105°C for 0, 0.5, 1, 2, 3, and 5 min. Spores suspended in de-ionized water (~1.4×10^8 CFU/ml) were used as control. Samples were processed using a high pressure microbial kinetic tester for two different pressurization rates (fast: 18.06 MPa and /s and slow: 3.75 MPa/s). Spore survivors were enumerated by pour plating, using Trypticase Soy Agar after incubation at 32°C for 48 h.

Results: Immediately after pressure come-up time, a slow pressurization rate reduced the population of *B. stearothermophilus* and *B. amyloliquefaciens* spores suspended in de-ionized water up to 6.8 and 2.5 log units, respectively. A slow pressurization rate further enhanced microbial lethality up to 2 min, but the effect disappeared after extended pressure holding times. Irrespective of the pressurization rates, *B. stearothermophilus* spores were reduced to below the detection limit (< 10 CFU/ml) after 1 min, whereas about 1.5 log units of *B. amyloliquefaciens* spores survived after 5 min PATP treatment. Both egg patty mince and tomato paste exhibited a protective effect, during extended pressure holding time (up to 5 min) in comparison to spores suspended in de-ionized water.

Significance: This study demonstrated that pressurization rate and food composition greatly influenced PATP spore inactivation.

P5-67 Influence of Minerals on Sporulation and Heat Resistance of *Clostridium sporogenes*

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Introduction: Clostridium sporogenes PA 3679, a non-pathogenic, putrefactive and spore-forming anaerobe, has been used as a surrogate to validate thermal sterilization processes. Although a simple and convenient sporulation medium has been used successfully for over 40 years to obtain *C. sporogenes* spores, using the medium to induce sporulation of vegetative cells is time consuming.

Purpose: This study was performed to investigate the effects of minerals on the degree and induction time of sporulation of *C. sporogenes* and to provide an alternative to reduce the time required for preparing spores.

Methods: A variety of mineral supplements were added to a basal medium in which sporulation of *C. sporogenes* vegetative cells occurs; the supplements used in this study were as follows: $CaCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_2$ and KCl to supply cation and $CaCl_2$, $CaCO_3$, $CaSO_4$, $Ca(OH)_2$ and $CaHPO_4$ to supply anion. Test cultures were periodically drawn for determination not only of viable counts of both spores and vegetative cells, but of heat resistance of the spores as well. Heat resistance of spore crops was also compared in large-scale studies.

Results: Of the cations tested, the addition of $CaCl_2$ caused a higher increase in spore number. The optimum concentration of $CaCl_2$ was 0.5%, which yielded nearly 10^4 CFU/ml of spores after 3 weeks. Of anions tested, $CaCO_3$ was found to promote sporulation markedly. With the optimized concentration of 0.5% $CaCO_3$, the spore amount obtained after one week was approximately 10^5 CFU/ml. The cultures produced by adding $CaCl_2$ and $CaCO_3$ showed no significant differences in heat resistance. D121°C values of the spore crops prepared with $CaCl_2$ and $CaCO_3$ in large scale studies were found to be 0.92 min.

Significance: These data suggest that $CaCO_3$ is highly effective not only in enhancing heat resistance, but in reducing sporulation time of *C. sporogenes*.

P5-68 Efficacy of Disinfection Methods of Decontamination of Infant Bottles Used for Feeding Powdered Formula Milk

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Introduction: Infant susceptibility to infections associated with bottle feeding with powdered formula milk has received increased attention in recent years because of contamination with pathogens such as *Enterobacter sakazakii* and *Salmonella*. Failure to adequately decontaminate used feeding equipment increases the risk of infections, and it is imperative that cleaning and disinfection procedures are effective.

Purpose: The aim of this study was to test the efficacy of four disinfection procedures, using feeding bottles that had contained reconstituted formula spiked with potential pathogens.

Methods: To represent potential microbial contamination of used feeding bottles, duplicate (n = 5) bottles of reconstituted formula were spiked with 10^5 CFU/ml mixed culture, 10^2 and 10^4 CFU/ml *Enterobacter sakazakii*, *Bacillus cereus* and *Staphylococcus aureus*. Before disinfection, bottles were cleaned according to recommended guidelines. Four disinfection procedures were tested, including a hypochlorite based chemical solution and three heat based methods – boiling, steaming and microwaving. Post-disinfection, each bottle was microbiologically sampled in four sites (inner screwcap, bottle interior, bottle outer rim and nipple interior).

Results: Prior to cleaning and disinfection, the inner screwcap and nipple interior were the most heavily contaminated sites, with $1.6–7.4 \times 10^3$ CFU/ml mixed

culture; the bottle interior was more contaminated overall, with 1.2×10^4 CFU/ml. Post disinfection, 800 sites were sampled and no *B. cereus* or *E. sakazakii* were isolated. *S. aureus* was isolated from 0.1% of sites with one site exceeding 1 CFU/ml – an inner nipple disinfected in a steam sterilizer.

Significance: Cumulatively, results from this study indicate slight differences in the disinfection ability of different methods. Nevertheless, strict adherence to recommended procedures (combined with adequate hygiene) will allow effective decontamination to be achieved. This highlights the importance for consumers and caregivers to strictly adhere to recommendations when preparing bottles for feeding.

P5-69 The Profiles of Tetracycline Resistance Bacteria in Human Microflora Associated with the Infant Digestive System

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Introduction: The rapid emergence of antibiotic resistant (ART) pathogens poses a serious health concern. A large antibiotic resistance (AR) gene pool has recently been found in commensal bacteria associated with many retail foods. The AR genes from the food isolates are functional and transmissible to human residential bacteria in laboratory settings. These foodborne ART bacteria can potentially serve as AR gene donors for horizontal gene transfer events and change the AR profiles of the human microflora through daily food consumption.

Purpose: The objective of this study was to investigate the development of AR in human gut microflora by analyzing the shift of ART bacteria in infant feces from newborn to 12 months of age.

Methods: The AR profile of the bacteria were assessed by (1) isolating ART bacteria by plating on antibiotic containing agar plates, followed by PCR confirmation of the presence of corresponding AR genes, and (2) quantify the tetM AR gene pool by real-time PCR.

Results: Tetracycline-resistant (Tetr) bacteria colonization started before solid food consumption in both breast- and formula-fed infants, shortly after birth, without the selective pressure of the corresponding antibiotics. The numbers of ART bacteria increased initially and was maintained throughout the study. Our data suggested that routes other than food may have played a role in the initial colonization of ART bacteria in infants.

Significance: Results from this study provide insights on the AR status in human gut microflora, and will aid in the identification of major routes of AR in humans.

P5-70 DSC Ability of Cleaners and Sanitizers to Degrade Curli Produced by Shiga Toxin-Producing *Escherichia coli*

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Introduction: Proteinaceous cell surface complements, curli, mediate adhesion and colonization of Shiga toxin-producing *E. coli* (STEC) to its contact hosts. They are also crucial components for biofilm formation on food-contact surfaces.

Purpose: The purposes of this study were to evaluate several enzymatic and chemical agents for their abilities to degrade curli, and to confirm the expression and degradation of curli protein by use of molecular techniques.

Methods: Three pairs of STEC, each having a curli-producing and a non curli-producing member, were treated with protease (1 unit or 2 units), acids (2% acetic acid or lactic acid) and detergents (Quorum Yellow or Zep formula 7961) under appropriate conditions. Curli protein from treated and untreated STEC cells was extracted and purified. The purified curli protein was quantified, and the identity of the protein was confirmed by amino acid sequencing and the efficacy of curli degradation by protein gel electrophoresis.

Results: The results showed that the curli protein expressed by the cells of STEC has a MW of ca 15-KDa. The treatments with protease reduced the overall mean amount of curli by 6.39–6.51%. Acetic and lactic acid at 2% concentration reduced the overall mean amount of curli by 1.45% and 15.43%, respectively. Use of 4% lactic acid further, but not significantly, reduced the amount of curli on the surface of STEC cells. Treatments with Quorum Yellow decreased the overall mean amount of curli by 75.75%, while the treatments with Zep formula 7961 reduced the overall mean amount of curli by 77.25%. Protein gel electrophoresis confirmed the results of curli degradation.

Significance: The treatments with protease, organic acids and detergents partially degraded curli protein on the surface of STEC cells. Some of the tested agents worked more effectively than others The study identified cleaning/sanitizing agents that can potentially be used to control biofilm in the food processing environment.

P5-71 Recovery of Viable Cells of Shiga Toxin-Producing *Escherichia coli* as Influenced by the Use of Different Neutralizing Solutions after Sanitizing Treatments

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Introduction: Organic acids and detergents are routinely used by the food processing industry for sanitizing food contact surface, equipment and utensils.

Purpose: The purposes of this study were to evaluate the efficacy of organic acids and commercial detergents in inactivating the cells of Shiga toxin-producing *Escherichia coli* (STEC), and of various neutralizing solutions in recovering viable cells of STEC after sanitizing treatments.

Methods: Cells of 12 STEC strains with various surface appendages were treated with acetic acid, lactic acid, Quorum Yellow and Zep formula 7961. After each treatment, the residual sanitizing agents in treated cell suspensions were neutralized with 4 different neutralizing solutions (Dey-Engley (DE), morpholinepropanesulfonic acid (MOPS), phosphate buffered saline (PBS) and sodium thiosulfate buffer, respectively) at room temperature for 10 min. The recovery of viable cells as influenced by the use of different neutralizing solutions was determined by growing the cells on Tryptic Soy Agar at 37°C for 24 h.

Results: Results indicated that treatments with organic acids and commercial detergents significantly ($P < 0.05$) reduced the populations of STEC. Treatments with Zep formula and Quorum Yellow were the most effective, reducing the overall mean populations of STEC cells by 8.78–8.99 $\log_{10}$CFU/ml. Treatments with acetic and lactic acid resulted in reductions in the overall mean populations of STEC by 0.35–1.85 and 1.93–3.36 log units/ml, respectively. The four neutral-

izing solutions were equally effective, and the different recoveries of viable cells of STEC following the sanitizing treatments ranged from 0.28 to 0.30 $\log_{10}$CFU/ml and were not statistically significant ($P > 0.05$).

Significance: The study suggests that treatments with Quorum Yellow and Zep formula were most effective, followed by treatments with lactic acid and then acetic acid. The four neutralization solutions can all be used for effectively recovering viable cells of STEC after sanitizing treatments. The DE buffer offered an indication of pH change, making it easier to use.

P5-72 Prevalence, Persistence, and Spread of *Listeria* spp. in a Commercial Delicatessen

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Introduction: Given the strong association between *Listeria monocytogenes* and deli meats, retail delicatessens continue to be one probable source for the spread of this pathogen.

Purpose: A long-term environmental survey was conducted at one retail deli to gather information on the incidence, spread and persistence of *Listeria*, including *L. monocytogenes*.

Methods: A total of 790 environmental samples were collected at a retail delicatessen during 14 alternating morning and afternoon monthly visits (~56 samples/visit) and analyzed for *Listeria,* using the standard USDA enrichment protocol. Sampling sites included floor drains, food preparation/contact surfaces, slicers, display cases, basement/kitchen walk-in coolers and sandwich lines. All *L. monocytogenes* isolates were typed by pulsed-field gel electrophoresis (PFGE) to assess strain persistence and possible contamination routes. In addition, an on-site *Listeria* control program was administered to upper management midway through the 14-month survey.

Results: Overall, 170 samples (21.5%) yielded *Listeria,* with 90 (11.4%), 50 (6.3%), and 30 (3.8%) of these samples containing *L. seeligeri, L. innocua* and *L. monocytogenes*, respectively. *Listeria* spp. were most frequently recovered from the basement (drains, floors in walk-in coolers) (29%) followed by kitchen (drains, floors in walk-in cooler) (21%) and sandwich lines (12%), with no significant difference between morning and afternoon samples ($P > 0.05$). Four persistent *L. monocytogenes* PFGE types were identified in the basement walk-in cooler and floor drain, kitchen floor drain and walk-in cooler and floor near display cases. Six non-persistent *L. monocytogenes* PFGE types were also recovered from the delicatessen slicers, kitchen walk-in cooler entry, and sandwich line floor. Several *L. monocytogenes* PFGE types spread from the basement to the kitchen. After introducing the *Listeria* control program, the overall incidence of *Listeria* spp. decreased 40%.

Significance: These findings demonstrate both the spread and persistence of *Listeria* and reinforce the need for *Listeria* control programs at retail delicatessens.

P5-73 Inactivation of Pathogens in Compost Mixtures as Influenced by Type of Manure

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Introduction: During aerobic composting, heat is generated from the metabolic activity of thermophilic microorganisms and may contribute to inactivation of contaminant pathogens at internal sites of static piles. At the surface of compost piles, however, heat dissipation contributes to reduced temperatures and in turn reduced pathogen inactivation. It is unknown whether pathogen inactivation at the surface would be affected by the compost composition and in particular the type of manure.

Purpose: To investigate the role of manure type on pathogen stability in compost mixtures.

Methods: Chicken, cow, and hog manures served as the source of nitrogen in compost mixtures while straw and cottonseed meal were used as carbon amendments. Mixtures varied in the C:N ratio, having initial values of 20:1, 30:1, or 40:1, and were inoculated with both gfp-labeled *Salmonella* spp. and gfp-labeled *Listeria monocytogenes*. Mixtures were placed in trays (simulating surface sites of static compost piles) and held in environmental controlled chambers at 20° or 30°C and under different levels of light exposure. On a weekly basis, moisture levels in samples were adjusted to initial values (30% or 60%). Samples were periodically taken for enumeration of pathogens and measurement of moisture and pH.

Results: At both 20° and 30°C, pathogen survival was greatest in compost mixtures formulated with cow manure, followed by mixtures formulated with chicken manure and then hog manure. Regardless of the manure used in the compost mixture formulation, however, *L. monocytogenes* populations decreased faster than *Salmonella* spp. populations. Exposure to conditions simulating bright sunlight accelerated pathogen inactivation.

Significance: Type of manure used in compost mixtures will affect the storage time necessary for complete inactivation of pathogens.

P5-74 Evaluation of Novel Antimicrobials to Inhibit the Growth of *Listeria monocytogenes* in Ready-to-Eat Boneless Ham and Uncured Turkey Breast

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Introduction: Listeria monocytogenes is a Gram-positive, non-spore-forming microorganism prevalent in the environment and often found on ready-to-eat meats. This microorganism causes severe illness when consumed by those at risk, and has been the subject of numerous RTE food recalls in recent years. *L. monocytogenes* is a critical threat to the food industry largely due to its ability to grow at refrigerated temperatures (4°C).

Purpose: The purpose of this study was to compare the inhibition of *L. monocytogenes* growth on fully cooked boneless ham and uncured turkey breast through the use of pioneering antimicrobials.

Methods: The performance of patented, proprietary blends of vinegar and lemon juice concentrate (MOstatin LV1), vinegar (MOstatin V), potassium lactate and vinegar (Opti.form Vinegar), buffered sodium citrate and sodium diacetate (IONAL LC- used only in boneless hams) was evaluated in whole muscle, boneless ham and uncured turkey breast for their ability to limit the growth of *L. monocytogenes* and extend shelf life when compared to control samples containing no antimicrobials. Each ham and turkey sample was surface inoculated with a four-strain cocktail of *L. monocytogenes* at 3-4 log CFU/g and 4–5 log CFU/g, respectively, then vacuum packaged and stored at 4°C. The samples were analyzed for *L. monocytogenes* populations by plating on Tryptic Soy Agar with a MOX overlay and aerobic plate counts (APC).

Results: MOstatin LV1 demonstrated ≤ 2.0 log CFU/g growth of *L. monocytogenes* in both test products. Additionally, MOstatin V also exhibited ≤ 2.0 log CFU/g growth of *L. monocytogenes* in the boneless ham.

Significance: These data suggest that natural blends of vinegar and lemon juice concentrate (MOstatin LV1) have a significant inhibitory effect on the growth of *L. monocytogenes* on fully cooked ham and uncured turkey breast while the natural blend of vinegar (MOstatin V) showed inhibitory effect in only the boneless ham.

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Adler, Jeremy M., Colorado State University (P4-35)
Algino, Ryan J., University of Wisconsin-Madison (P2-61)
Al-Sakkaf, Ali, Massey University (P4-59)
Arthur, Lindsay, Ontario Ministry of Agriculture, Food and Rural Affairs (T3-01)
Badvela, Mani Kumar, National Center for Food Safety and Technology (P2-69)
Ball, Brita, University of Guelph (P3-64)
Barmpalia-Davis, Ioanna M., Colorado State University (P2-56)
Bhagat, Arpan R., Purdue University (P1-36)
Björnsdóttir, Kristín, North Carolina State University (T3-04)
Blessington, Tyann, University of California–Davis (P1-32)
Borowski, Alena G., University of Wisconsin-Madison (P2-45)
Buchholz, Annemarie L., Michigan State University (T4-11)
Caballero Vidal, Cesar, Kansas State University (T5-01)
Carlson, Brandon A., Colorado State University (T1-11)
Carter, Kristina K., University of Tennessee (T1-08)
Chaney, William E., Texas Tech University (P1-24)
Chapman, Benjamin, University of Guelph (T6-03)
Cheon, Jeong-Hwan, Konkuk University (P3-39)
Critzer, Faith J., University of Tennessee (T1-01)
DeDonder, Sarah, Kansas State University (T6-07)
Derevianko, Alexandra M., University of Delaware (T1-07)
Diop, Michel Bakar, Agricultural University of Gembloux (P5-53, P5-54)
Dirks, Brian P., Drexel University (P2-44)
Dominguez, Silvia A., Rutgers, The State University of New Jersey (P2-65)
Drake, Stephenie, North Carolina State University (T3-06)
Dwivedi, Hari Prakash, North Carolina State University (P3-32)
Esquivel, Julian J., Universidad Autonoma de Queretaro (P1-20)
Fernandes, João, Universidade Católica Portuguesa (P5-38)
Franco, Wendy, University of Florida (P5-13)
Fry, Pamela, The Ohio State University (P4-53)
Girard, Maryline, Université Laval (P4-71)
Gragg, Sara E., Texas Tech University (P1-26)
Han, So-Ri, Konkuk University (T5-07)
Harper, Nigel, Kansas State University (T3-09)
Hirneisen, Kirsten A., University of Delaware (T2-12)
Ijabadeniyi, Oluwatosin, University of Pretoria (P1-45, P2-19)
Ivy, Reid A., Cornell University (T1-09)
Jagadeesan, Balamurugan, Purdue University (P3-49)
Jones, Joseph M., The Ohio State University (P2-03)
Kakani, Grihalakshmi, Texas A&M University (P2-67)
Kanayeva, Damira, University of Arkansas (P4-24)
Kannan, Aditi, Washington State University (P2-66)
Kim, Meebok, The Ohio State University (P3-63)
Kim, Kyeongyeol, Gyeongsang National University (P1-47)
Kumar, Saurabh, University of Nebraska-Lincoln (P2-09)
Kwon, Kyung Yoon, Kyung Hee University (P1-40)
Lansdowne, Lancya, West Virginia University (P5-65)
Laury, Angela, Texas Tech University (P2-41)
Laycock, Andrea, University of Delaware (P3-23)
Lee, Jae-Hoon, Konkuk University (P3-15)
Lee, Hyo-Won, Gyeongsang National University (P1-66)
Li, Xinhui, The Ohio State University (P2-18)
Li, Min, University of Arkansas (T4-05)
Li, Xiaojing, The Ohio State University, Dept. of Food Science and Technology (P5-01)
Lopez-Velasco, Gabriela, Virginia Tech (P1-22)
Luciano, Fernando B., University of Manitoba (T2-01)
Manuzon, Michele Y., The Ohio State University (T5-12)
McKinney, Julie S., Virginia Tech (P4-51)
Min, Kyung Jin, Kyung Hee University (P5-06)
Mohamed, Tagelsir, University of Maryland Eastern Shore (T3-07)
Montes, Alejandrina, Universidad Autonoma de Nuevo Leon (P5-47)
Morandage, Janaka S., Purdue University (P4-11)
Mudoh, Meshack, University of Maryland Eastern Shore (P5-57)
Neetoo, Hudaa S, University of Delaware (P5-62)
Niode, Omar T., University of California, Davis (P3-53)
Obaidat, Mohammad M., University of Georgia (T2-10)
Pagadala, Sivaranjani, University of Maryland Eastern Shore (P5-63)
Palmer, M. Elizabeth, Cornell University (T1-04)
Pan, Wenjing, Rutgers, The State University of New Jersey (P5-15)
Park, Jung-Youn, Konkuk University, College of Veterinary Medicine (T5-06)
Park, Yoen Ju, The University of Georgia (P5-70)
Parry-Hanson, Angela, University of Pretoria (P4-36)
Pathanibul, Panchalee, University of Tennessee (P2-08)
Pedigo, Ashley, University of Tennessee (P3-58)
Perry, Jennifer, The Ohio State University (P2-70)
Petrova, Vera, University of Vermont (P3-52)
Platt, Tammy M., West Texas A&M University (T2-03)
Randhawa, Suneet, Texas Tech University (P4-15)
Richardson, Arena N., University of Georgia (P4-62)
Saini, Jasdeep K., Kansas State University (T6-09)
Sanchez, Maria D., New York State Dept. of Agriculture & Markets (P4-45)
Sasanya, Stella Opendi, North Dakota State University (P3-66)
Sasanya, James J., North Dakota State University (T3-02)
Seo, Eunkyoung, Gyeongsang National University (P3-50)
Shen, Cangliang, Colorado State University (P2-50)
Simpson, Catherine A., Colorado State University (P2-47)
Singla, Anika, University of California–Davis (P3-29)
Solis, Luisa, Universidad Autonoma de Nuevo Leon (P4-03)
Syne, Stacey-Marie, The University of the West Indies, St. Augustine (P2-53)
Tabe, Ebot, North Dakota States University (T5-05)
Tadesse, Daniel A., The Ohio State University (T1-12)
Theofel, Christopher, University of California, Davis (P1-08)
Tiao, Narry, The Ohio State University (P2-25)
Valderrama, Wladir B., Pennsylvania State University (P2-39)
Valtierra, Diana, Universidad Autonoma de Nuevo Leon (P5-36)
Weber, Casey G., Kansas State University (T2-02)
Wei, Jie, University of Delaware (T1-06)
Weinkauf, Heidi, Iowa State University (P5-51)
Whitney, Brooke, North Carolina State University (P5-59)
Wiegand, Kimberly M., University of Wisconsin-Madison (P2-31)
Xiao, Linlin, The Ohio State University (P4-28)
Xu, Hua, Kangwon National University (P4-56)
Yoo, Byong Kwon, The University of Georgia (P4-39)
Yuan, Wei, The Ohio State University (T6-01)
Yun, Hyun Sun, Korea University (P4-46)

PROGRAM ADDENDUM

as of July 25, 2008

95th Annual Meeting
IAFP 2008
Columbus, Ohio • August 3–6

MONDAY MORNING — AUGUST 4

PROGRAM ADDITION

10:30 a.m. **Food Safety: How FSIS Uses Risk and Risk Assessments to Make Public Health Decisions**
Union A–C

SCOTT HURD, Deputy Undersecretary for Food Safety, USDA-FSIS, Washington, D.C.

S3 **Globalization of Acceptance Criteria for Microbiological Methods: Separating the Science from the Politics**

9:30 a.m. Title Change — Viewpoint: Worked on ISO Regulations for Method Approvals. Represents the European Point-of-View. To Discuss Changes in Regulations in Europe and the Role of European Commission — ROY P. BETTS, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, UK

RT1 **Eating Seafood — Is It Worth the Risk?**

9:20 a.m. Title Change — Sushi Done Right is Safe to Eat — JOE HUNSAKER, Hissho Sushi, Charlotte, NC, USA

P1 **Produce, Toxicology and Sanitation Poster Session**

P1-46 Withdrawn

MONDAY AFTERNOON — AUGUST 4

S4 **Bacterial Physiology — A Forgotten Theme That is Critical for the Food Microbiologist**
Sponsored by ILSI North America Technical Committee on Food Microbiology

CHANGE OF PROGRAM

1:30 p.m. Genomics Meets Physiology: What Have Genomics Taught Us about the Effects of Growth Phase and Stress Exposure on Bacterial Physiology? — CHARLES W. KASPAR, University of Wisconsin-Madison, Madison, WI, USA

2:00 p.m. How the Physiological State of the Challenge Inoculum Affects Validation Study Outcomes — LARRY R. BEUCHAT, University of Georgia, Center for Food Safety, Griffin, GA, USA

2:30 p.m. Effects of Growth Phases, Temperature and Stress Exposure on Foodborne Pathogen Virulence: The *L. monocytogenes* Example — KATHRYN J. BOOR, Cornell University, Dept. of Food Science, Ithaca, NY, USA

3:00 p.m. Break

3:30 p.m. Effects of Growth Phases, Temperature and Stress Exposure on Foodborne Pathogen Survival and Stress Resistance: The *Salmonella* Example— ROY P. BETTS, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, UK

4:00 p.m. Effects of Stress Exposure on Foodborne Pathogen Physiology: The *E. coli* Example — TERESA M. BERGHOLZ, Cornell University, Dept. of Food Science, Ithaca, NY, USA

4:30 p.m. Development and Validation of Detection Methods — How Does the Physiology of the Target Cell Affect Assay Sensitivity? — MARTIN WIEDMANN, Cornell University, Dept. of Food Science, Ithaca, NY, USA

S7 **Food Safety Issues in Food Transportation — Keeping It Cold and Keeping It Clean**
Sponsored by the IAFP Foundation

2:00 p.m. Speaker Change — RFID: New Applications in the Food Industry — will be presented by MAGALIE LANIEL, University of Florida, Gainesville, FL, USA

RT2 **Occurrence and Control of Norovirus: Is Public Vomiting Public Enemy #1?**
Union A-C

CHANGE OF PROGRAM

1:30 p.m. Recommendations and Questions of Clean-Up and Liability of Noroviruses — MELVIN KRAMER, EHA Consulting Group, Inc., Baltimore, MD, USA

1:50 p.m. Influence on Genotypes on Emerging and Increasing Norovirus Virulence and Infectivity — JAN VINJE, CDC, Atlanta, GA, USA

2:10 p.m. Perspectives on Battling and Combating Norovirus Infections — HAL KING, Chick-fil-A Restaurants, Atlanta, GA, USA

2:30 p.m. Roundtable Discussion on Survival and Control of Noroviruses on Fomites — CHARLES GERBA, University of Arizona, Tucson, AZ, USA

2:45 p.m. Roundtable Discussion

P2 **Meat and Poultry, Microbial Food Spoilage, Beverage and Dairy Poster Session**

P2-28 Withdrawn

P2-53 Detection and Identification of *Listeria* spp. at DSC Different Processing Stages of Ready-to-Eat Meat Products Sold in Trinidad — STACEY-MARIE SYNE, Adesh Ramsubhag and Abiodun Adesiyun, The University of the West Indies, La Romain, Trinidad

Abstract correction – *Listeria* is one of the most important pathogens found in food and infections via this organism accounts for nearly 500 deaths in the US each year.

TUESDAY MORNING — AUGUST 5

RT4 **Global Perspectives and Novel Approaches for Effective Food Safety Communication within Culturally Diverse Audiences**

Sponsored by the IAFP Foundation

9:15 a.m. Speaker Change — Reaching Consumers in an Asian Environment — will be presented by ANDY BENSON, International Food Information Council, Washington, D.C., USA

T4 **Risk Assessment and Produce Technical Session**

T4-05 Presenter Change — Predictive Modeling of *Listeria monocytogenes* Reduction on Fully-Cooked Chicken Drumettes DSC during Post-Process Hot Water Pasteurization — will be presented by LISA COONEY, University of Arkansas, Fayetteville, AR, USA

T4-07 Presenter Change — Combined Effects of Sucrose Laurate Ester and Pressure-Assisted Thermal Processing to Inactivate *Bacillus amyloliquefacien* Spores Suspended in Mashed Carrots — will be presented by W. RATPHTAGSANTI, The Ohio State University, Columbus, OH, USA

P3 **Applied Laboratory Methods, Education and Epidemiology Poster Session**

P3-19 Evaluation of ChromID *sakazakii* Medium (ESPM) for the Recovery of *Enterobacter sakazakii* from Several Food and Environmental Samples — J. M. ROCHE, I. Desforges, L. Restaino and F. Villeval, bioMérieux, Inc., Salins, France address correction Rue des Aqueducs, 69290 Craponne

P3-24 Withdrawn

P3-65 Misspelling in Title — Determining the Level of Compliance with Legal Traceability Requirements — GORDON HAYBURN and Andrew Clarke, The Tetley Group Limited, Greenford, Middlesex, UK

P3-69 Presenter Change — Evidence for Implicating Food Vehicles in Outbreaks, 1998–2006 — will be presented by TRACY AYERS, CDC, Atlanta, GA, USA — Author List Correction — Tracy Ayers, Julian Grass, Mike Lynch and Ian Williams

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TUESDAY AFTERNOON — AUGUST 5

S13 **Pathogen Data Sharing to Advance Food Safety**

Franklin A-C

2:30 p.m. Speaker Change — Data Sharing, a Critical Component for Developing Effective International Food Safety Risk Management Metrics — ROBERT L. BUCHANAN, DHHS/FDA/CFSAN, College Park, MD, USA

S14 **Food Safety and Regulatory Issues Associated with Non-Thermal Processing of Foods and Beverages**

Union A-C

Sponsored by the IAFP Foundation

2:30 p.m. Speaker and Title Change — Pulsed Electric Fields and Ultrasound Processing — will be presented by DANIELA BERMUDEZ-AGUIRRE, Washington State University, Pullman, WA, USA

4:30 p.m. Speaker Change — Panel Discussion member JOHN B. DUBECK, Keller and Heckman, LLP, Washington, D.C., USA will replace David R. Joy, Keller and Heckman, LLP, Washington, D.C.

S15 **Harmonization of Irrigation Water Practices**

Sponsored by the IAFP Foundation

2:30 p.m. Non-Microbial Threat Analysis of Irrigation Water — To be determined.

P4 **Pathogens and Novel Laboratory Methods Poster Session**

P4-05 Withdrawn

P4-57 Misspelling in title — Simple, Rapid and Reliable Detection of Enterohemorrhagic *Escherichia coli* O26 Using Immunochromatography — TARO YONEKITA, Tatsuya Fujimura, Takashi Matsumoto and Fumiki Morimatsu, Nippon Meat Packers, Inc., Tsukuba, Ibaraki, Japan

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WEDNESDAY MORNING — AUGUST 6

S17 **Dairy Pasteurization in Today's Risk-Based Food Safety Environment — International Perspectives on the Use of Risk Assessment Tools**

ROOM CHANGE— location is now in Union A-C

Late Breaking Session – Tomatoes, Peppers, Cilantro? Consequences of the *Salmonella* Saintpaul Produce-Related Outbreak

Union A-C

Organizers: Gary Acuff and Alejandro Castillo

Convenors: Gary Acuff and Alejandro Castillo

8:30 a.m. Details of the Epidemiological Investigation — IAN WILLIAMS, CDC-NCZVED, Atlanta, GA, USA

9:00 a.m. Regulatory Perspective on Recent Outbreak and Investigation — SHERRI MCGARRY, FDA, College Park, MD, USA

9:30 a.m. Research Update — KEITH SCHNEIDER, University of Florida, Gainesville, FL, USA

10:00 a.m. Break

10:30 a.m. The View from Mexico and Current Research — CRISTOBAL CHAIDEZ-QUIROZ, Centro de Investgacion en Alimentacion y Desarrollo, Culiacan, Sinaloa, Mexico

11:00 a.m. Impact and Response from the US Industry — DAVE GOMBAS, United Fresh Produce Association, Washington, D.C., USA

11:30 a.m. Roundtable Discussion and Questions Including all speakers and PATTY LOVERA, Food & Water Watch, Washington, D.C., USA

RT5 **Comparative International Approaches to Regulating Unsafe Food**

CHANGE OF PROGRAM

9:15 a.m. The Finnish/EU Legal Approach to Managing Unsafe Food — PEKKA PAKKALA, Finnish Food Safety Authority Evira, Helsinki, Finland

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RT6 **Water: Potability vs. Drinkability**
Delaware A-D
Sponsored by the IAFP Foundation

CHANGE OF PROGRAM

11:30 a.m. Roundtable Discussion

WEDNESDAY AFTERNOON — AUGUST 6

S22 **What is the "Real" Issue with MDR?**

2:30 p.m. MDR from across the Pond: The United Kingdom View — To be determined.

S23 **The Greening of Food Packaging — Safety of Biodegradeable, Reused and Recycled Food Packaging**

CHANGE OF PROGRAM

1:30 p.m. Current Technologies for Recyclable, Reuseable and Biodegradable Food Packaging – SUSAN SELKE, School of Packaging, Michigan State University, East Lansing, MI, USA

1:55 p.m. Recycling and Reusing Plastic Milk Bottles – EDWARD KOSIOR, Nextek Limited, London, UK

2:20 p.m. Industry Experience with Recycling Composite Food Packaging Material – Representative from TetraPak, Inc., Columbia, MD, USA

2:35 p.m. Industry Experience with Safety of Biodegradable Food Packaging Material – LARRY FOX, NatureWorks LLC

2:55 p.m. Risk Analysis of Food Safety Issues Related to the Recycling, Reuse and Biodegradability of Food Packaging Material – MICHAEL C. VAN DERVEER, FDA, College Park, MD, USA

3:15 p.m. Panel Discussion

T7 **Spoilage and Epidemiology Technical Session**

T7-05 Increasing Incidence of Listeriosis in France and Its Relations with Host Factors and Food Control — ALEXANDRE LECLERCQ, Alban Le Monnier, Marc Lecuit and Véronique Goulet, Institut Pasteur, Paris, France

Abstract correction – the results should read "from 4.6 cases per million inhabitants in 2006 to an estimated value of 5.0 in 2007."