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IVAN PARKIN LECTURE ABSTRACT

It's the Science, Stupid

Reflections on 41 Years as a Food Microbiologist

Presented by

Mr. Carl Custer
Food Microbiologist
Bethesda, Maryland

Scientific discovery has been the basis for our advancements in public health, welfare and the many conveniences of modern life. In the past millennium, especially in the past century, science has provided a food supply that is both more abundant and safe. But human nature has remained the same.

In my 41 years as a food microbiologist, I have observed that ignorance, laziness and even greed have caused many foodborne outbreaks. The past year's headlines show human nature continues to foster outbreaks. It's not just the producers and processors, but also policy makers who have let problems slide because the epidemiology did not support action (too few bodies). I will give a few examples.

Almost 400 years ago, Sir Francis Bacon, cribbing from earlier philosophers, wrote, "For what a man had rather were true he more readily believes. Therefore he rejects difficult things from impatience of research... things not commonly believed, out of deference to the opinion of the vulgar."

As food science professionals, we can provide the science to prevent or minimize future outbreaks. While we do not have all of the answers, we have many of them. We must explain and articulate the scientific issues to the farmers producing our food, to the government policy regulators, to industry's marketers and processors and to the politicians providing funds and drafting laws. We can use the science and the rules of logic to overcome the prejudices and beliefs of those with the power to affect food safety. It's good for business, it's good for public health, it's good for the "General Welfare."

Also, 400 years ago, William Shakespeare wrote, "Ignorance is the curse of God, knowledge, the wing wherewith we fly to heaven." Let's fly.

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JOHN H. SILLIKER LECTURE ABSTRACT

Trends in Food Safety Management

Presented by

Dr. Terry A. Roberts
Food Safety Consultant
Reading, United Kingdom

Despite the widespread implementation of HACCP and a range of Good Practices (Veterinary, Hygienic, Manufacturing), and greatly improved controls during food processing and distribution, in some countries much reliance is still placed on microbiological criteria. The purposes, and limitations, of microbiological criteria will be reviewed briefly, and recent changes in the application of microbiological criteria in the European Union described. Despite modelling microbial death having been accepted for decades in thermal processing, modelling microbial growth and survival in foods was met with suspicion, even hostility. The development of adequate and useful models of growth will be summarized. The urgent need for an accessible, and relevant, database of microbiological data and information for food technologists and regulators will be restated. Some unresolved problems in food safety management will be discussed, including the value of challenge testing, psychrotrophic saccharolytic anaerobes in ready-to-eat foods and risk-based decisions.

SYMPOSIUM ABSTRACTS

RT1 Using HACCP to Innovate New Processes in Retail Food Operations

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The FDA Retail Food Code says that, if the retail food industry wants new processes, it must innovate them using HACCP. It is not the FDA's responsibility to develop new processes. Of course, new process innovation is at the heart of a strong retail food industry. IAFP's retail food members constantly seek ways to improve their processes, and they need knowledge about HACCP technology and process development. While many operators know about the seven principles of HACCP, these principles are merely steps in a development process and do not provide information about scientific development – specifically, hazard identification, development and validation of controls, development of monitoring and corrective action procedures, and finally, verification procedures. On the other hand, numerous studies in the *Journal of Food Protection* have discussed new process validation (e.g., safety of rice cakes; using buckets of vinegar solution to rinse fingers). Presentations 1, 2, and 3 will provide information about the science of retail cold holding, pasteurization, and cooling, which are crucial to most safe retail food processes. In Presentation 4, a grocery company representative will discuss the use of HACCP science to validate and implement new process controls in grocery stores. Presenters will then ask questions of each other and participants as to how they can use the information presented in this roundtable to innovate new processes when they return to their operations.

RT2 The Management and Control of Chemical Hazards in Food

P. MICHAEL BOLGER, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; DAVID F. KENDRA, USDA-ARS-NCAUR, 1815 N. University St., Peoria, IL 61604, USA; LARRY KEENER, International Product Safety Consultants, 4021 W. Bertona St., Seattle, WA 98199, USA; MARK MOORMAN, Kellogg Company, Porter Street Office Complex, 235 Porter St., Battle Creek, MI 49014, USA; JEFFREY A. KEITHLINE, Keller and Heckman LLP, 1001 G St. NW, Suite 500 West, Washington, D.C. 20001, USA; PETER J. SLADE, National Center for Food Safety and Technology, 6502 S. Archer Road, Summit-Argo, IL 60501, USA; JON DEVRIES, Medallion Labs/General Mills, 9000 Plymouth Ave. N., Minneapolis, MN 55427, USA; TONG-JEN FU, FDA, National Center for Food Safety and Technology, 6502 S. Archer Road, Summit-Argo, IL, 60501, USA; KATHLEEN A. LAWLOR, PepsiCo, 100 Stevens Ave., Vallhalla, NY 10595, USA

Recently there appears to have been more publicity of the risks associated with particular chemical hazards in certain foods than in previous years. There are a number of reasons for this, not least of which is the fact that new concerns have arisen in areas least expected to garner such attention, and also as a result of contemporary electronic dissemination of information and reaction to such episodes through the Internet. Issues such as food allergens, heavy metal contamination of seafood and candies, acrylamide formation, and pesticide and other residue contamination, have all been featured in the popular press and debated via the web. There have been corresponding responses from regulatory agencies, the industries affected, the scientific community, as well as from consumers and other stakeholders. This roundtable proposes to consider and reflect on some of the major issues and events chronicled over the past few years, and to bring particular focus on the management and control of the events in question, through scientific and/or, regulatory means with the associated communication of messages to stakeholders regarding the apparent risk(s). The roundtable will be organized to allow recognized subject-matter experts to give the background on recent major events and to consider the key requirements for effective control of the respective issues through communication of core scientific and regulatory principles. In addition to those speakers giving introductory addresses regarding some of the more notable cases, other speakers will consider the control of chemical hazards implicated in such events through application of HACCP, and the efficacy of messages relating to relative risk as communicated to consumers.

RT3 Water Emergencies: Too Much, Too Little, Too Late and What is the Plan?

CHUCK GERBA, University of Arizona, College of Agricultural Life Sciences, Bldg. 90, Tucson, AZ 85721-0038, USA; RICHARD GELTING, CDC, National Center for Environmental Health, 4770 Buford Highway NE, Atlanta, GA 30341-3724, USA; MICHAEL BRODSKY, Brodsky Consultants, 73 Donnamora Cresent, Thornhill, ON, L3T 4K6, Canada; J. ALAN ROBERSON, American Water Works Association (AWWA), 1300 Eye St. NW, Suite 701 W, Washington, D.C. 20005, USA; SUSAN MCKNIGHT, Quality Flow Inc., 3691 Commercial Ave., Northbrook, IL 60062, USA; PETER KENNEDY, Quality Flow Inc., 3691 Commercial Ave., Northbrook, IL 60062, USA; DEAN DAVIDSON, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740, USA

An "emergency" is defined as a sudden, usually unexpected occurrence or occasion requiring immediate action. Disasters occur when hazards strike in vulnerable areas. With less than 1% of the earth's water supply being made up of freshwater, any dramatic change in it affects the availability of ingredient water for the food supply. Suddenly having too much water, cutting off a water supply for an extended period of time or chronic water shortages with no end in sight; what can or should to be done to ensure the quality and quantity of ingredient water under these conditions? This roundtable will focus on both the short and long term impact of water emergencies on the quality and quantity of ingredient water for use in the food supply. Speakers will share their insight on the impact of water shortages on irrigation waters use for product production, how Hurricane Katrina's damage to the drinking water systems and sewage systems crippled foodservice operations attempting to open, the role of the public health department in water disasters like the *E. coli* tragedy in Walkerton, Ontario, Canada, and the impact from loss of water service from a boil water incident in Cary, NC will be discussed. Questioners will be from industry and government, and audience participation will be encouraged. The Water Safety & Quality PDG will then develop a white paper from the roundtable discussion with the hope of publication in *Food Protection Trends*.

RT4 With Over 100 Years of Experience in Food Safety, We Think...

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Food has likely never been as safe as it is today, but it remains a significant public health issue. Each year it is estimated that one-third of individuals in developed countries become ill from food and water each year. From the origins of food preservation to modern interventions, there have been advances and impediments in food protection. This symposium poses the question: "What is the best, and conversely, the worst thing that has happened to food safety?" Each speaker will be given creative liberty to use their experience in the field and their unique vantage point to address this question. This symposium uniquely takes advantage of the expertise of these speakers who have significantly contributed to advancing food safety worldwide. This format allows speakers to share their thoughts and opinions with the membership of IAFP, especially those still in graduate school or just starting their careers. This is an informal discussion that acknowledges past accomplishments and challenges but also describes changes in food safety that have occurred or that will occur on a global scale. This discussion is intended to stimulate conversation amongst the IAFP membership to encourage a comprehensive approach to addressing and accomplishing food safety objectives. This symposium aims to generate discourse on innovative approaches to reduce the burden of foodborne illness and to encourage members to view food safety as a dynamic field that requires awareness of all perspectives.

RT5 Panel on the Science Behind Temperature Control of Potentially Hazardous and High Risk Food

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Potentially hazardous foods (PHFs) traditionally are foods that are capable of supporting growth of infectious or toxigenic microorganisms. These foods are typically prone to temperature abuse and should be kept at temperatures that prevent microbial growth (e.g., < 4°C – > 60°C). However, there are exceptions that do not fit the definition precisely. The 2005 version of FDA Model Food Code has an extensive section on a revised definition of PHFs, based on work by the Institute of Food Technologists (IFT), who reviewed the evolution of the term PHF, and recommended a change to time/temperature

control for safety (TCS). The IFT developed a framework that could be used to determine whether a food is a PHF (TCS food) or not. Part of the framework includes two tables that consider the interaction of pH and a_w in a food, whether the food is raw or heat treated, and whether it is packaged. When further product assessment is required, the application of microbiological challenge testing (inoculation studies) is discussed along with pathogen modeling programs and reformulation of the food. This new definition is considered by some to be overly complicated for food operations like retail stores and restaurants to follow. The roundtable will hear the rationale for the development of the new definition and how the food industry and states that adopt the Code will react. Questioners will be from other national jurisdictions as how their countries respond to this in comparison with their definitions and management policies.

RT6 Food Safety Laws: Political Science or Food Science

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The United States Government Accountability Office recently recognized food safety as a high priority area for the federal government. This follows a year of high profile foodborne disease outbreaks which has prompted a call for review and perhaps revision of our national approach to regulatory oversight. Although most food safety laws originated over 100 year ago, but many changes have occurred in the farm-to-fork continuum since that time.

In February 2007, members of Congress introduced the Safe Food Act, an initiative intended to consolidate food safety efforts at the federal level by the creation of a single Food Safety agency. Specifically, the Safe Food Act would merge the food safety related activities of at least eight different federal agencies, with the new agency directed by a single administrator. The legislation proposes new regulations that would require the following: (1) registration and regular inspection of food processors; (2) oversight of process control systems in food establishments; (3) standards and regular sampling/testing for food contaminants; (4) regular inspection of and uniform standards for imported foods; (5) a national foodborne disease surveillance program overseen by the US Centers for Disease Control and Prevention; and (6) ongoing research and education to improve food safety. This initiative is consistent with those of many other nations which have already consolidated their food safety efforts.

A panel has been convened to discuss the pros and cons of this proposal. The panel consists of individuals representing the following perspectives: the National Academy of Sciences IOM report: Ensuring Food Safety from Production to Consumption; the World Health Organization; the food industry; and a former US food safety regulator. Following brief presentations by the panelists, there will be a panel discussion with questions and answers from the audience. The goal of this roundtable is to promote open communication between the various constituents as we seek for continued improvement in food safety using a science-based approach.

S1 Foodborne Disease Update

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The Foodborne Disease Update Symposium provides attendees with updates on significant foodborne disease outbreak investigations and other related foodborne disease issues. This year's symposium will cover two themes: The major *E. coli* O157: H7 outbreaks in the US linked to spinach and lettuce and the multiple outbreaks of botulism in the US and Canada linked to carrot juice from a California processor.

S2 Vaccination Strategies to Control Foodborne Pathogens from Farm-to-Table

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In view of pressure to reduce antimicrobial drug usage and to emphasize preventive strategies to control foodborne disease transmission, there has been increased interest in vaccination as a means of protecting the public from foodborne disease. Vaccination strategies to prevent foodborne disease transmission provide unique challenges, though. For example, while the goal of traditional vaccination strategies often is to prevent disease, vaccination of farm animals to control foodborne disease often needs to prevent colonization with an agent that may not even cause disease in the host species vaccinates (such as *Escherichia coli* O157:H7 in cattle). This symposium will provide an overview of vaccination strategies that can help control foodborne disease from farm-to-table, including vaccination of farm animals that may be a reservoir for a foodborne pathogen as well as vaccination of food handlers and consumers.

S3 Food Defense Research and Application

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Developing a national research plan for food defense requires cooperation among federal and state public health agencies, academia, and industry. Several large research programs have been initiated in support of food defense. This symposium will include presentations on food defense research in government and academia. Presenters will describe their research programs, project future research needs, and provide highlights of recent research discoveries. Select scientists will share development and validation of detection methods for high consequence microbes, toxins, and chemicals in food matrices. Rapid detection of potential pathogens and toxins in the food system from pre farm inputs through final consumption represents a significant challenge due to the range of potential agents, the complex matrices in which they may be present and the environments where the testing needs to be conducted. Studies determining the viability/survivability of threat agents in food and accompanying models for pathogen risk management will also be presented. In addition, this symposium will provide information on how government and industry are using food defense research findings to inform their activities, including the development of countermeasures, such as guidance documents, testing for threat agents, tabletop exercises and training activities.

S4 Outreach Programs to Promote Dairy Products and Their Safety around the World

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In developed nations, advanced systems are in place to assure the safety of foods which move through global commerce. This is not the case in developing nations, where infant mortality, starvation and disease result from lack of availability of food. A variety of federal and private sector partnerships are working to assist and enhance dairy quality and safety in less industrialized regions of the world. Efforts around the globe are underway to reinterpret traditional practices, resulting in improved microbiological safety. Some of these efforts are utilizing sophisticated information technology to deliver scientific information on-site to remote locations around the globe. This symposium will showcase efforts underway in the Middle East, India and Africa which will enhance the biodiversity of traditional dairy foods while offering substantial public health protection. This symposium will also remind us of the need to share our vast knowledge and resources with less fortunate societies to improve food safety for all.

S5 Measuring and Motivating Safe Food-handling Practices at Home, Retail and Food Service

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One of the challenges in food safety is the dissimilarity between the food handlers' knowledge of a recommended risk-reduction step and actual

practice. Differences between knowledge and practice can be found throughout the food production chain, from farm-to-fork. This symposium will provide the latest evidence-based information affecting the behavior of food handlers and preparation staff in retail, food service and home settings. Home food preparers are bombarded with sometimes inconsistent food safety information from a variety of sources thus providing a barrier to food safety risk communicators. The first portion of the symposium will center on exploring the factors that may contribute to differences between knowledge and practice in the home. Demographics, psychographics and task complexity impose challenges to the retail and food service sector with relation to food safety communication and resulting practices. The second segment of the symposium will focus on strategies, interventions and compelling tools directed at this segment. The final segment of the symposium will concentrate on verification, evaluation and measurement of the efficacy of risk communication strategies on targeted groups. Methodologies that seek to verify appropriate execution of recommendations will be identified throughout the symposium. This discussion aims to help reduce the gaps between food safety science, social science and risk reduction strategies with front-line food handlers within the risk analysis paradigm.

S6 Long-term Sequelae of Pathogens Transmitted by Food

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Diarrhea is often considered the form of clinical illness most associated with foodborne transmission of infectious agents. However, some pathogens transmitted by food can cause non-diarrheal illness, and some infectious diarrheal illnesses can have long-term sequelae, often of a non-enteric nature. Recent assessments of the burden of foodborne disease in the United States have focused on acute illness to measure morbidity, mortality and lost productivity. Less is known about the disease burden of post-infectious complications in defined populations. This symposium will provide an overview of long-term sequelae associated with recognized foodborne pathogens including *Campylobacter*, *Escherichia coli* O157:H7 and *Salmonella*. The possible association of foodborne infection with inflammatory bowel disease will be reviewed. For the most common bacterial foodborne pathogens, the economic costs of their long term sequelae will be discussed and compared to the costs of acute illness. The aim of the symposium is to provide a more complete perspective on the burden of foodborne disease in order to improve analyses of prevention efforts.

S7 The DaVinci Code of Auditing: Reaching the Holy Grail of One Global Standard

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Most food processors today are faced with undergoing a seemingly unending series of food safety audits – from direct customers, brokers, retailers, and foodservice clients. These audits can take the form of independent third party audits, specific client audits, and contractor audits. A single client may even request more than one type of audit at the same facility. Why is this? Is it possible to reach the elusive "Holy Grail" of one global food safety audit standard for food processing facilities? This symposium will explore that question from several perspectives. Having performed a number of audits for different clients, an independent contractor will present the differences and similarities between them, and give an opinion as to the potential of reaching a single harmonized standard. Progress on the acceptance of FPA SAFE audits will be discussed, as these were designed to be the single standard for food safety audits. Various other perspectives on harmonization and standardization of audits will also be presented by representatives from the retail and foodservice sectors, the Food Safety Leadership Council, SQF, and the British Retail Consortium. Having one single harmonized global food safety audit standard may not be as unreachable as we think, and may even easier to find than the Grail.

S8 Recent Pivotal Decisions of the National Conference on Interstate Milk Shipments

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The National Conference on Interstate Milk Shipments (NCIMS) and their Grade A milk safety program as practiced in all 50 States and Puerto Rico has kept Grade A milk and milk products safe for over 50 years. The struggle to fulfill their mission to

provide "The Safest Milk Supply for All the People" continues. Recently the NCIMS has made precedent setting pivotal decisions that are broad in scope with potential impact on and information for food regulation far beyond the dairy industry. This symposium is about some of those decisions. Topics will include: The NCIMS and their decision making processes. This presentation is intended to provide a context for the other presenters. The ongoing international pilot program for the inspection and IMS rating of milk plants and farm groups outside of the United States. This presentation will be given by a State milk safety regulator, either the Co-chair of this NCIMS Committee, or by one of the most active of the group members and may provide insight into the impact of, and one NCIMS response to, international trade agreements. How grade A aseptic milk will be regulated has been a subject of significant controversy over the last four years. This presentation will explore that controversy and the solution passed by the May 2007 NCIMS. The NCIMS HACCP Program Hazard Guide and the "pop-up" audit report forms are practical HACCP innovations that may be useful well beyond NCIMS Dairy HACCP. Paperless computer gathering, storage and reporting of critical pasteurization safeguard monitoring, raw milk storage and equipment cleaning. Other NCIMS Actions that may be critical. This presentation will cover other pivotal decisions of the May 2007 NCIMS that were not (and can not) be anticipated at the time of the writing of this proposal.

S9 What's the Future of Foodborne Pathogen Detection?

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The trend continues toward the use of more sensitive and rapid screening methods for the presence of foodborne pathogens in foods. The possibility of performing pathogen testing on-site, in food-processing facilities, has positive financial implication for the industry. Real time PCR is sensitive enough to enable quantitation of RNA from a single cell. Microarrays are useful when one wants to survey a large number of genes quickly or when the sample to be studied is small. Biosensors, which refer to chemical sensors in which the recognition element is a biochemical recognition system, bring unique opportunities to the area of rapid detection of foodborne pathogens. The combination of a short to no-sample preparation, a short enrichment procedure (if any at all), high throughput and a short time for the availability of the results make biosensors interesting candidates for the development of new platforms in the area of food safety. The complex nature of many sample matrices as well as the presence of particulate matter in samples may hinder the sensitivity and specificity of new detection systems, especially those that rely on immunochemical or nucleic acid technologies. In addition, regulatory agencies considered the results from these tests presumptive, and require that the organism be isolated as proof of contamination. The objective of the proposed symposium is to introduce some of the cutting-edge research in the area of new detection technology of foodborne pathogens including viruses to the different IAFP audiences. The limitations of these technologies and the promises that they can deliver in the context of food microbiology will be discussed in the presentations.

S10 The Impact of Emerging Food Trends on Food Safety

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Over recent years, the increased diversity of the population, along with changing consumer expectations for culinary variety, access to seasonal fresh foods year-round, and the desire for more simplistic, healthful food choices, has created new and unique challenges for food safety professionals. The symposium will begin with an overview of these emerging food trends from a consumer perspective. The remainder of the symposium will focus on the food safety implications of these trends with emphasis given to an increasing demand for natural ingredients and foods, the growth of organic offerings, probiotics, preferences for diverse, exotic, fresh foods, and the issues and strategies they have prompted within the food industry. The impact of food manufacturing using minimal and non-traditional processing methods to meet consumer expectations will also be examined.

S11 Food Allergies: A Growing Food Safety Concern

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An estimated 12 million Americans suffer from food allergies, an immune-system response to a food protein. Food allergy is continuing to increase and has become a food safety and public health concern resulting in 30,000 emergency room visits and 150–200 deaths each year. This symposium will include an update on recent research providing prevalence, fatality, research for a cure, consumer allergen labeling behavior and causes of reactions. A speaker from the Food and Drug Administration (FDA) will give an update on regulations from the US Food Code and state regulatory activity. This will also include information on results from the recent FDA Food Allergen Labeling Study. The symposium will provide insight to the impact of food allergies on the retail food industry. A retail food industry representative will discuss trends in the market place regarding consumer queries and expectations and share strategies for accommodating food allergic consumers and conveying allergen information in a restaurant setting. A packaged food industry representative will talk about trends in the packaged food industry including consumer calls, consumer behavior when allergens are added to a product and changes in formulations to accommodate allergens. Representatives from each industry will address best practices and what they are doing for managing allergens including test kits, education and training, signage at point of purchase and cooperative programs with non profits. A corporate lawyer specializing in food law will talk about compliance with statutes, regulations and guidance documents and provide insight into the most common causes of product liability cases for manufacturers and retail food service.

S12 The Wrath of Vibrio's "Past, Present and Future"

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Every year during the summer months illness associated with consumption of contaminated oysters are constantly making headlines. There is a need to understand how these outbreaks occur and what can be done to prevent them. This symposium will address the recent outbreaks of *Vibrio parahaemolyticus*, both nationally and internationally, what environmental factors have contribute to the increase in the population of these pathogens and what can be done to prevent outbreaks in the future. Additionally, what post-harvest remediation may lesson the risk assoc-iated with aquatic pathogens will be addressed. The participants will get an overview of the regulations associated with vibrios in shellfish and what new strategies are being developed to control vibrios in seafood. °Regulatory requirement in closing marine shellfish harvest areas are complex and inconsistent from state to state and internationally. This symposium will also cover topics associated with the mandatory closing of estuaries and halting harvesting shellfish. The strategies to open the affected waters to assure health safety and regulations surrounding the closing and opening will be presented.

S13 Pre-harvest Food Safety: Another Critical Consideration for Assuring the Safety of the Food Supply

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Over recent years, food safety professionals have increasingly referred to three major phases of the farm-to-fork continuum, i.e., pre-harvest (on farm), post-harvest (at processing and in distribution), and retail/home. A large proportion of the food safety regulations that have been promulgated in the last several decades have a specific focus on the second or post-harvest phase. Nonetheless, many foodborne pathogens enter the food supply during pre-harvest or production stages. This pre-harvest phase is sometime overlooked, but nonetheless, there are a large number of researchers who are engaged in pre-harvest food safety research, addressing issues such as tracking foodborne pathogens on the farm; understanding microbial diversity and evolution; elucidating mechanisms of antibiotic resistance and its transfer; and designing mitigation strategies to prevent or reduce pathogen contamination at the farm level. Many pre-harvest projects are large, multi-disciplinary endeavors that capitalize on the skills of microbiologists, epidemiologists, and veterinarians, with the cooperation of the production agriculture sector. This symposium provides the attendee with an introduction to the many concerns related to pre-harvest food safety and highlights recent efforts in pre-harvest food safety research. As with post-harvest and retail/consumer initiatives, pre-harvest efforts also seek to reduce foodborne disease and improve public health as a consequence of preventing contamination with foodborne pathogens. Educating food safety professionals about on-farm issues will provide a forum whereby all three sectors of the farm-to-fork continuum can come together in an effort to solve significant and complex food safety problems.

S14 Critical Issues in the Investigation of Outbreaks of Foodborne Illness Involving Food Workers

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The Committee on the Control of Foodborne Illness has, over the past three years, been working on a global analysis of the implication of infections of food workers and how their handling practices contribute to foodborne illness outbreaks. The purpose of this symposium is to discuss the role of the food worker and how well outbreaks are investigated. An extensive analysis of the worldwide literature indicates there are several key issues for workers, e.g., pathogen, food involved, carrier status and handling practices. Practically every outbreak investigated has one or more food worker errors. There will be a presentation on the ones where infected workers contribute to illnesses, and how they are characterized and controlled. The methods and extent to which outbreaks are investigated may vary considerably. The UK and US approaches to outbreak investigation are compared at the national and state level. Outbreak investigations involve a range of agencies and interested parties. While having a common public health goal, they may have competing priorities. Communication during an outbreak is one example where tensions can occur, especially in high profile cases involving the media. Another key issue is the correct sampling, testing, transport and laboratory procedures for the human and food specimens for outbreak management and for any ensuing legal proceedings. Following an outbreak a business may face a number of legal challenges and claims. Problems can occur if the business has ceased operations, and one example of a *E. coli* outbreak is discussed. A scientific, audit based approach to overcome this is described using case study material. This symposium should be of interest to anyone working in the food industry or enforcement.

S15 Balancing Cultural and Religious Norms and Food Safety

JOE REGENSTEIN, Cornell University, Dept. of Food Science, 116 Stocking Hall, Ithaca, NY 14853-7201, USA; SYED RASHEEDUDDIN AHMED, Muslim Consumer Group, P.O. Box 8538, Rolling Meadows, IL 60008, USA; GIHAN ELGINDY, Transcultural Education Center, P.O. Box 3292, McLean, VA 22103, USA; KIMBERLY LIVSEY, FDA-ORA-Southeast Region, 60 Eighth St., NE, Room 209, HFR-SE14, Atlanta, GA 30309, USA; CINDY JARDINE, University of Alberta, 551 General Services, Edmonton, AB, T6G 2H1, Canada

This symposium is intended to identify food preparation and consumption guidelines and requirements of the various cultural/religious groups including Jewish Kosher, Muslim Halal and Hindu food laws, as well as their origin. This information will be analyzed and compared to common food safety harvesting, processing, packaging, and final preparation laws and regulations in the Western World. The purpose is to determine if there are similarities and conflicts between these cultural/religious food requirements of certain populations and governmentally enforced food safety laws and regulations. Once the speakers have identified the similarities and conflicts between cultural/religious food requirements and governmental food safety requirements, a dialogue can begin to propose solutions to any conflicts or differences in order to reduce or remove regulatory challenges by governmental field inspectors and negative impacts on various cultural/religious groups.

S16 Microbial Biofilms and Biofilm Control

ETHAN B. SOLOMON, DuPont Chemical Solutions Enterprise, P.O. Box 80402, Wilmington, DE 19880, USA; AMY C. LEE WONG, University of Wisconsin-Madison, 1925 Willow Drive, Madison, WI 53706–1187, USA; BASSAM A. ANNOUS, USDA-ARS-ERRC, Food Safety Intervention Technologies Research Unit, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; DONALD W. SCHAFFNER, Rutgers University, The State University of New Jersey, Food Risk Analysis Initiative, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA; MICHAEL P. DOYLE, University of Georgia, Center for Food Safety, Griffin, GA 30223-1797, USA; DALE A. GRINSTEAD, Johnson Diversey, 8310 16th St., P.O. Box 902, Sturtevant, WI 53177-0902, USA

A biofilm is generally defined as an assemblage of bacterial cells adherent to each other and to an inert or living surface, and are enclosed in a protective layer of self-produced exopolymers. Bacterial cells within a biofilm present in the food handling environment or on food products were shown to be resistant to inactivation by cleaners, sanitizers, and disinfectants. This symposium will discuss the nature of biofilm formation in general and the effects that growth in a biofilm can have on bacterial cells. The presence of biofilms on food handling and processing equipment will also be discussed as will biofilms that can be found growing directly on food, especially fresh produce. Since, pathogenic microorganisms can form biofilms in food handling environments, it is important to control these biofilms. Ways to remove biofilms will be discussed including traditional cleaners, tools, and mechanical means to eliminate microbial communities. Also, non-traditional methods for controlling biofilms; including competitive exclusion, will be discussed. The final discussion will cover techniques to measure and manage biofilm control in food handling settings.

S17 Lettuce and Leafy Greens: Issues, Actions, and Opportunities

THAI-AN NGUYEN, CDC, Epidemiology Branch, Div. of Foodborne, Bacterial and Mycotic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Mailstop A-38, Atlanta, GA 30333, USA; MAHA HAJMEER, California Dept. of Health Services, Emergency Response Unit, Food and Drug Branch, 1500 Capitol Ave., P.O. Box 997435, MS-7602, Sacramento, CA 95899-7435, USA; DAVID E. GOMBAS, United Fresh Produce Association, 1901 Pennsylvania Ave. NW, Suite 1100, Washington, D.C. 20006, USA; CHUCK GERBA, University of Arizona, College of Agricultural Life Sciences, Bldg. 90, Tucson, AZ 85721-0038, USA; RUTH L. PETRAN, Ecolab Inc., 655 Lone Oak Drive, Eagan, MN 55121, USA; SHIRLEY B. BOHM, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740 USA

Lettuce and leafy greens products have been epidemiologically linked to numerous multi-state *E. coli* O157:H7 foodborne illness outbreaks in the last decade. In the fall of 2006 foodborne illnesses associated with consumption of spinach and shredded lettuce captured nationwide consumer, industry, media and regulatory attention. These outbreaks associated with consumption of lettuce and leafy greens products were a watershed moment in the public debate regarding how best to assure the safety of fresh lettuce and leafy greens products as well as all fresh produce. This symposium will review CDC epidemiological data as well as FDA foodborne illness outbreak investigation findings regarding these food borne illness outbreaks. Industry actions that have been subsequently implemented to enhance the safety of lettuce and leafy greens production, processing, distribution and retailing will also be discussed. In addition, alternative approaches to assuring the safety of fresh and fresh-cut lettuce and leafy greens products will be discussed in relation to the role that time/temperature for safety plays as well as microbial testing. Produce food safety research priorities will also be discussed in light of the information presented.

S18 Preparing Scientists for the Legal Aspects of a Crisis: Step into an Interactive Mock Trial and Learn How to Become an Expert Witness

DONNA GARREN, National Restaurant Association, 1200 17th St. NW, Washington, D.C. 20036, USA; DAVID E. ERNEST, Bullivant, Houser, Bailey, Attorneys at Law, 888 S.W. Fifth Ave., Suite 300, Portland, OR 97204-2089, USA; TONI HOFER, Raley's & Bel Air, 500 W. Capitol Ave., West Sacramento, CA 35605, USA; TBD

Understanding the science behind the crisis is only the first step in becoming an expert witness in a foodborne illness trial. Preparing to testify can be a crisis in itself, if you're not prepared. This interactive symposium will teach attendees how to give credible answers and project a positive professional image as an expert witness in a court case. Guidance from professional attorneys and scientists during a mock trial will demonstrate the proper tips and techniques for you to use when giving a deposition or testimony. This hands-on approach will highlight the importance of presenting accurate information while using the right language. After the mock trial scientists – from all industry segments – and lawyers will participate in a round table discussion, share personal experiences and answer questions from the attendees.

Learn how to ...

- Handle difficult questions
- Present information relating to preventing a foodborne crisis from occurring
- Discuss the benefits of a food safety training program
- Develop key phrases and priority messages relating to foodborne illness crisis
- Be confident and prepared

S19 Applications of "omics" Technologies for Food Safety and Security

BART WEIMER, Utah State University, 4700 Old Main Hill, Logan, UT 84322-4700, USA; ANDREW K. BENSON, Univesity of Nebraska-Lincoln, Dept. of Food Science and Technology, 330 Food Industry Complex, Lincoln, NE 68583-0919, USA; AVRAHAM RASOOLY, NIH-NCI and FDA-CDRH, 6130 Executive Blvd., Room 6035, Rockville, MD 20852, USA; SOPHIA KATHARIOU, North Carolina State University, Dept. of Food Science, Campus Box 7624, Schaub Hall, Room 322A, Raleigh, NC 27695-7624, USA; F. CHRIS MINION, Iowa State University, Dept. of Veterinary Microbiology and Preventive Medicine, 1130 Veterinary Medicine, Ames, IA 50011-1240, USA; DARRELL O. BAYLES, USDA-ARS, National Animal Disease Center, 2300 Dayton Road, Ames, IA 50010, USA

Food safety and food security are important global issues. Research employing "omics" technologies, including genomics, proteomics, and metabolomics, is helping to elucidate pathogen behavior at the molecular level and to develop better detection and typing systems. "Omics"-based tools enable researchers to explore complex biological processes in a quantitative and integrative manner via a systems biology approach. These methods of analysis are helping to identify genes that are potential targets for interventions, genes that play a role in pathogenesis, and genes that are responsible for specific survival and virulence characteristics. The availability of completely sequenced genomes of foodborne pathogens has made possible the analysis of these genomes solely using computational methods. Microarray-based comparative genomics research, which takes advantage of information available from whole genome sequences, is leading to an increased understanding of the evolution and pathogenesis of foodborne pathogens and is providing critical information for the development of improved detection and genotyping methods, as well as for intervention strategies for the control of foodborne pathogens. Used as diagnostic tools, DNA micro-arrays offer the capability to detect a broad spectrum of pathogens simultaneously in a relatively short period of time and can be used in instances of possible bioterrorism. Techniques that fall within the category of either proteomics or protein arrays are used for global analysis of cellular protein output under different conditions, including those in food environments. The integration of both genetic- and protein-based analyses provides a more comprehensive view of cellular activities. Thus, the application of "omics" technologies will play an important role in understanding how pathogens survive food safety barriers and interact with host species and will ultimately give rise to better tools for control.

S20 Food Safety @ the Speed of Thought – Creating Virtual Networks

Nutcracker 3

Organizers: Gary Acuff and Frank Yiannas

Convenors: Gary Acuff and Frank Yiannas

Presenters to be determined

Food safety awareness is at an all time high and new and emerging threats to the global food supply are being recognized. Achieving food safety success in this changing environment requires going beyond traditional approaches to food safety. It requires greater collaboration than ever before.

In today's modern environment, with increased disbursement of team members — whether globally or domestically — it has become more critical than ever to leverage certain forms of technology in order to collaborate on ideas, documents, and decision-making across distance. In addition to understanding the technology tools available, research shows that about 50% of virtual teams fail to attain their goals, because of difficulties related to building trusting, positive relationships across the boundaries of geography, time zones and cultural differences.

By attending this session you'll hear firsthand from Information Technology experts about technological tools available to create virtual teams as well as tips on how to use these tools to enhance your ability to communicate with colleagues across distance.

S21 Spoilage and Its Control in Meat Products

ROBIN KALINOWSKI, NCFST, 6502 South Archer Road, Summit-Argo, IL 60501, USA; RICHARD HOLLEY, University of Manitoba, Dept. of Food Science, Winnipeg, MB, R3T 2N2, Canada; DARREN CORNFORTH, Utah State University, 8700 Old Main Hill, Logan, UT 84322, USA; JEFF KORNACKI, Kornacki Microbiology Solutions, LLC, 6308 Mourning Dove Drive, McFarland, WI 53558, USA

The meat industry is one of the largest segments of the food processing industry that continues to deal with the issue of meat spoilage as an economic loss. The spoilage of meat is dependent on the nature of the product and handling of the product from production, through the chill chain, to the consumer. Approaches to control spoilage of meat and meat products can range from good manufacturing practices and chill chain management to the application of novel antimicrobials. This symposium will address microbiological and non-microbial spoilage of meats and will include examples of case studies in troubleshooting and control of microbiological spoilage of meats.

S22 Mitigating Spoilage Risks in Ready-to-Drink Beverages

SEAN LEIGHTON, The Coca-Cola Company, One Coca-Cola Plaza, 409A USA, Atlanta, GA 30313, USA; TIM GUTZMANN, Ecolab Inc., Research Center, 655 Lone Oak Drive ESC-F52, Eagan, MN 55121, USA; CATHY MOIR, Food Science Australia, P.O. Box 52, North Ryde, NSW, 1670, Australia; KEIICHI GOTO, Mitsui Norin Co. Ltd., Food Research Laboratories, 223-1, Miyahara, Fujieda, Shizuoka, 426-0133, Japan; HISATO IKEMOTO, Suntory Ltd., Beverage and Food Division, 57, Imaikami-cho, Nakahara-ku, Kawasaki, Kanagawa, 211-0067, Japan

Ready-to-Drink (RTD) beverages are those that need no preparation by the consumer. Even if chilled products are excluded from this category, we are still left with a continually expanding array of products – from traditional carbonated drinks to shelf-stable juice products, bottled water, "near water"/flavored water products, health and sports drinks, energy drinks, and an extensive range of tea and coffee drinks. Each of these relies on some form of antimicrobial treatment to provide shelf stability and safety – whether by physical processes, such as heat, carbonation, microfiltration UV or ozonation or by hurdles such as pH, sugars, potassium sorbate or similar. With new products entering the market, using ingredients from various and sometimes novel sources, we can expect new challenges for product formulators, process engineers and microbiologists. The spoilage microorganisms we thought we had to control have increased in number as the categories of RTDs and ingredients broaden – from conventional yeasts, molds and lactics, our attention moved to *Gluconobacter, Acetobacter, Byssochlamys* and other heat-resistant molds, preservative munching *Zygosaccharomyces bailii*, to alicyclobacilli and even more recently to sporolactobacilli – microorganisms that were not known to exist when many shelf-stable technologies were developed. This symposium on RTD beverages will provide an update on some of the spoilage microorganisms, their sources, their detection from the myriad of other microbes present in many raws and process environments, designing challenge studies that show how robust our products are, using plant sanitation and equipment design, all helping to mitigate the risks of microbial spoilage.

S23 Emerging Issues Affecting Dairy Product Quality and Safety

MARIANNE SMUKOWSKI, Wisconsin Center for Dairy Research, Babcock Hall, 1605 Linden Drive, Madison, WI 53706, USA; KATHRYN J. BOOR, Cornell University, Food Science Dept., 413 Stocking Hall, Ithaca, NY 14853, USA; KRISTEN DIXON, Chestnut Labs, 3233 E. Chestnut Expressway, Springfield, MO 65802, USA; JOHN LARKIN, FDA, 6502 S. Archer Road, Summit-Argo, IL 60501, USA

This symposium will educate attendees on scientific studies and regulatory activities that are currently impacting food safety and quality of dairy products. Available information on the bacterial pathogen growth, stasis and death in cheeses that assists in determining what storage temperatures are necessary to maintain product safety will be reviewed. Research revealed that the inherent characteristics of most cheeses create a hostile environment for bacterial pathogens, especially at elevated ripening and storage temperatures. The second presentation will cover HTST fluid milk spoilage profiles, response of psychrotolerant endospore-forming bacteria to processing temperatures and sources of these microbes in milk processing systems. The presence of *Bacillus* and *Paenibacillus* spp. in HTST pasteurized fluid milk has emerged as a key hurdle in extending product shelf lives beyond 14 days. Recent collaborative work completed on a quantitative method for the rapid detection of organisms in several dairy products will be covered in the third presentation. The TEMPO system utilizes a 16 tube MPN test and can significantly decrease the time for test results compared to the traditional MPN test. Block and shred cheeses, milk powders and cheese seasonings were compared to the traditional plate count and MPN tests to evaluate reproducibility, repeatability and labor savings. The final presentation will be on the regulatory perspective of past pasteurization requirements and current pasteurization technologies. Information will be presented on the National NACMCF definition of pasteurization and how the FDA is addressing the NACMCF recommendation. The concept of risk assessment will also be included.

Interactive Session: A Mystery Outbreak —What to Do When It Happens to You!

Representatives from:

CDC
Central Florida Chapter of the American Red Cross
FBI – Florida Branch
FDA, Southeast Regional Office
FDA–Emergency Coordination and Response Team at CFSAN
Florida Dept. of Agriculture and Consumer Services
Florida Dept. of Health, Bureau of Community Environmental Health
Florida Division of Emergency Management
Florida Hospital Association, Health Care Research and Information Services
Office of Richard T. Crotty, Mayor, Orlando, Florida
Orange County Florida EMS, Office of the Medical Director
Orange County Florida Fire Rescue
Orange County Florida Health Department
Orange County Florida Sheriff's Office
Sanitation and Safety Inspections, Dept. of Business and Professional Regulations, Division of Hotels and Restaurants
University of Florida, Center for Food Distribution and Retailing

To present, in an informational, up-to-date scenario, the depiction of a mysterious outbreak that occurs at an imaginary resort, that uses a real-time, fresh and interactive format. The purpose is for IAFP members to participate from, see from, think from and act from a different perspective than is their common, day-to-day role in the matter of food safety and food security. We want to promote cross-disciplinary thinking. We will offer fresh perspectives from expert-in-field speakers who are responsible, on a daily basis, for the response to the presence of an outbreak. Ultimately, the goal is to provide IAFP members with a real-life circumstance that they interact with and witness how an outbreak occurs. We intend the participants go back to their respective positions with a new found appreciation for what they contribute to the world of food safety and security.

Special Interest Session: *Salmonella* Growth, Persistence and Survival in Low-moisture Foods and Their Environments—Strategies for Control

Nutcracker 3

ROY BETTS, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD, UK; ANN MARIE MCNAMARA, Silliker Inc., 160 Armory Drive, South Holland, IL 60473, USA; PAUL HALL, ConAgra Foods, Inc., Center for Research Quality & Safety, Six ConAgra Drive, Omaha, NE 68102, USA; BILL PURSLEY, AIB, 123 Bakers Way, P.O. Box 3999, Manhattan, KS 66505-3999, USA; DON ZINK, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA

The recent recall of peanut butter underscores that there is much to learn about the ecology of this organism, especially its survival in the environment and its routes of transmission other than fecal contamination.

It appears that the organism does not have to grow in a food product to cause infection, that the infectious dose in many cases is low, and that certain foods can protect this organism in its passage through the gastrointestinal tract.

How do we prevent environmental contamination from this organism, particularly in environments where wet sanitation is contraindicated?

What can we learn from recent outbreaks of *Salmonella*, what do we know about the ecology of this organism and where are our gaps in knowledge?

How do we take the science, our collective knowledge and make it actionable in manufacturing?

This session will bring into sharp focus our collective knowledge in this area and best practices on controlling *Salmonella* in reduced moisture products.

ILSI North America Symposium Series

Monday Morning, July 9

8:30 a.m. – 12:00 p.m.

S02 Symposium on Vaccination Strategies to Control Foodborne Pathogens from Farm-to-Table

Co-convenors: Joseph D. Meyer and Martin Wiedmann

In view of pressure to reduce antimicrobial drug usage and to emphasize preventive strategies to control foodborne disease transmission, there has been increased interest in vaccination as a means of protecting the public from foodborne disease. Vaccination strategies to prevent foodborne disease transmission provide unique challenges, though. For example, while the goal of traditional vaccination strategies often is to prevent disease, vaccination of farm animals to control foodborne disease often needs to prevent colonization with an agent that may not even cause disease in the host species vaccinates (such as *Escherichia coli* O157:H7 in cattle). This symposium will provide an overview of vaccination strategies that can help control foodborne disease from farm-to-table, including vaccination of farm animals that may be a reservoir for a foodborne pathogen as well as vaccination of food handlers and consumers.

8:30 Vaccines against Diarrheal and Foodborne Diseases — An Overview — KAREN L. KOTLOFF, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St., Baltimore, MD 21201, USA

9:00 Efficacy of a Vaccine Product Containing Type III Secreted Proteins for Reduction of *Escherichia coli* O157:H7 in Cattle — RODNEY A. MOXLEY and David R. Smith, Dept. of Veterinary & Biomedical Sciences, University of Nebraska, Lincoln, NE 68583-0905, USA

9:30 Controlling *Salmonella* through Vaccination in Chickens — Charles Hofacre and JOHN J. MAURER, Dept. of Population Health, The University of Georgia, 953 College Station Road, Athens, GA 30602, USA

10:00 Break

10:30 Vaccination of Food Service Workers as an Intervention Strategy against Foodborne Diseases: The Hepatitis A Example — RYAN T. NOVAK, Epidemiology Branch, Division of Viral Hepatitis, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS G-37, Atlanta, GA 30333, USA

11:00 Vaccinating Humans against *Campylobacter* — ANTHONY P. MORAN, Laboratory of Molecular Biochemistry, Department of Microbiology, National University of Ireland — Galway, University Road, Galway, Ireland

11:30 Current Status of Norovirus Vaccine Development — ROBERT L. ATMAR, Departments of Medicine and Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS BCM 280, Houston, TX 77030, USA

12:00 Adjourn

Vaccines against Diarrheal and Foodborne Diseases — An Overview

KAREN L. KOTLOFF, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St., Baltimore, MD 21201, USA

Foodborne diseases represent a worldwide health threat, regardless of a country's level of economic development. Vaccines offer one of the most effective and economical adjuncts to public health measures for the control of these illnesses, as illustrated by the success of poliovirus and hepatitis A virus vaccines. The decision to develop a vaccine involves consideration of the disease burden, the feasibility of developing a safe and effective vaccine, and the commercial viability of the product. Theoretical issues, practical considerations, and preclinical experiments are used to determine the optimal approach (e.g., inactivated whole cells, live attenuated, and subunit vaccines), as well as the route of administration, adjuvant, and delivery system to be pursued. Often a strategy is selected that mimics protective immune responses that occur in nature.

If a vaccine appears safe and efficacious in pre-clinical trials, the path to licensure involves serial Phase 1, 2 and 3 clinical trials to evaluate its safety, immunogenicity, and efficacy in humans. Simultaneously, an economically viable mechanism must be established for ensuring adequate supply and distribution of high quality vaccine. Many vaccines against foodborne diseases are in development or licensed. Some vaccines against infections deemed to be high public health priorities, such as rotavirus, cholera, and typhoid fever, have reached licensure, while others, such as *Shigella* and enterotoxigenic *Escherichia coli* vaccines, remain in clinical trial. Progress has been made in pre-clinical and some clinical trials of candidate vaccines against numerous other foodborne pathogens, including *Campylobacter jejuni*, *Salmonella* Paratyphi A and B, *Clostridium difficile*, enteropathogenic *Escherichia coli*, enterohemorrhagic *E. coli*, noroviruses, *Entameba histolytica*, *Cryptosporidium*, and botulism.

Efficacy of a Vaccine Product Containing Type III Secreted Proteins for Reduction of *Escherichia coli* O157:H7 in Cattle

RODNEY A. MOXLEY and David R. Smith, Dept. of Veterinary & Biomedical Sciences, University of Nebraska, Lincoln, NE 68583-0905, USA

Escherichia coli O157:H7 is an important zoonotic foodborne and environmental pathogen; preventing infection in cattle has been proposed to reduce the risk of human disease. A bacterial extract vaccine (Bioniche Life Sciences, Belleville, Ontario) containing *E. coli* O157:H7 type III secreted proteins was tested in a series of vaccine trials in beef feedlots. We tested the efficacy of vaccination on *E. coli* O157:H7 colonization of the terminal rectum mucosa (TRM), fecal shedding, hide contamination and pen-level contamination resulting from natural exposure. In the 2003 trial, the efficacy of 1, 2, or 3 doses of the vaccine based on fecal shedding was 68, 66, and 73% respectively, compared with pens of nonvaccinates. In a 2004 trial, cattle given 3 doses were 98.3% less likely to be colonized than nonvaccinates. In a 2004 large-scale trial (n = 20,556), pens of cattle given 2 doses were 27% less likely to test positive for exposure than pens of nonvaccinates. In a subset (n = 720), vaccinates had a 75% lower probability of being colonized. In a 2005 trial, cattle in vaccinated regions of the feedyard were 62% and 54% less likely to shed *E. coli* O157:H7 in their feces and have hide contamination, respectively, compared to cattle in nonvaccinated regions. In a 2006 trial, a 3-dose regimen had 63% efficacy based on fecal shedding. The results indicate this vaccine has the potential to be an effective preharvest intervention method to prevent cattle from being colonized with *E. coli* O157:H7, and prevent subsequent contamination of feces and hides and further environmental transmission.

Controlling *Salmonella* through Vaccination in Chickens

Charles Hofacre and JOHN J. MAURER, Department of Population Health, The University of Georgia, 953 College Station Road, Athens, GA 30602, USA

Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP), implemented in 1997, set *Salmonella* performance standards for the meat-processing industry in an attempt to reduce salmonellosis finally in the US (5). Initially, significant progress was made in reducing the level of carcass contamination with *Salmonella* to 9%. Unfortunately, *Salmonella* prevalence in processed meats has steadily "inched" closer to the 20% limit set by FSIS. We have been working with one poultry integrator that has failed HACCP *Salmonella* performance sets. We implemented a *Salmonella* vaccination program at the broiler-breeder level for this company, using a combination of live and killed *Salmonella* vaccines to cover the prominent *Salmonella* serovars identified in their performance sets. An oral, live *Salmonella* Typhimurium vaccine, MeganVAC1, was given to pullets followed by a bacterin vaccine, consisting of *S.* Kentucky and *S.* Berta. We monitored several pullet flocks for *Salmonella*, followed them to broiler-breeder farms, and followed their progeny to broiler farms and ultimately, the processing plant. We collected relevant epidemiological data including *Salmonella* prevalence at the plant. Results were compared to those collected for a second poultry integrator, not vaccinating the birds against *Salmonella*. Vaccination did not appear to reduce *Salmonella* prevalence at broiler-breeder or broiler level compared to the unvaccinated control group. However, the poultry integrator was able to reduce *Salmonella* prevalence at the plant to meet HACCP *Salmonella* performance standards. We are presently typing *Salmonella* to serovar/strain level to see what impact vaccination has on *Salmonella* serovar distribution within this poultry integrator.

Vaccination of Food Service Workers as an Intervention Strategy against Foodborne Diseases: The Hepatitis A Example

RYAN T. NOVAK, Epidemiology Branch, Division of Viral Hepatitis, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS G-37, Atlanta, GA 30333, USA

Hepatitis A is caused by hepatitis A virus (HAV). Transmission occurs by the fecal-oral route, either by direct contact with an HAV-infected person or by ingestion of HAV-contaminated food or water. Foodborne outbreaks typically are associated with contamination of food during preparation by an HAV-infected food handler. Although food handlers with hepatitis A are frequently identified (7% of case-patients identified through sentinel surveillance 2001–2006 reported being a food handler), outbreaks are relatively uncommon in the United States since the majority of food handlers with hepatitis A do not transmit HAV. However, when infected food handlers are identified, evaluation of the need for immunoprophylaxis and implementation of control measures are a considerable burden on public health resources. Food handlers are not at increased risk for HAV infection because of their occupation and, therefore, are not currently recommended for vaccination by the Advisory Committee on Immunization Practices. In the event of a common-source exposure where a food handler receives a diagnosis of hepatitis A, immune globulin (IG) should be administered to other food handlers at the same establishment. Because common-source transmission to patrons is unlikely, IG administration to patrons typically is not indicated but may be considered in specific circumstances.

Reducing foodborne transmission of HAV can be achieved by improving food production and food handler hygiene and by providing preexposure prophylaxis to persons at risk for infection. Food

handlers acquire HAV infection from others within their communities, and reducing foodborne transmission of HAV will be achieved ultimately through routine vaccination of persons at risk for HAV infection within these communities.

Vaccinating Humans against *Campylobacter*

ANTHONY P. MORAN, Laboratory of Molecular Biochemistry, Dept. of Microbiology, National University of Ireland — Galway, University Road, Galway, Ireland

Campylobacter jejuni is the leading cause of bacterial enteritis in humans and is associated with the development of the post-infectious sequelae of reactive arthritis and the paralytic disorder Guillain-Barré syndrome (GBS). The economic burden of acute *C. jejuni* infection is exacerbated by GBS, particularly requiring intensive care. Infection by *C. jejuni* is waterborne or foodborne mainly, and undercooked or contaminated poultry are implicated particularly. As the development of an anti-*C. jejuni* vaccine would be a major asset to control this infection, especially in traveller's diarrhea, the aim of this report is to review progress in the field and the problems to be overcome. Since there are a variety of *C. jejuni* serotypes that can vary between outbreaks and traveller's diarrhea attention has focused previously on the use of an oral, inactivated, whole-cell vaccine expressing conserved antigens. Although human volunteer studies have been conducted (Phase I and II), the potential for the induction of anti-*C. jejuni* antibodies cross-reactive with neuronal gangliosides, implicated in GBS development, has led to discontinuation of this vaccine. Further studies have examined the potential of a recombinant truncated flagellin subunit vaccine, a multivalent vaccine expressing *Campylobacter* antigens, and live, attenuated *Salmonella* Typhimurium vectoring *Campylobacter* antigens as candidate strategies in animal studies. Moreover, a proteomic-based approach has been applied to the identification of cell surface proteins of potential use as recombinant vaccine candidates. The performance of challenge studies in humans, nevertheless, is complicated by the need for selection of non-GBS-inducing challenge strains. Collectively, the vaccine studies that have been conducted in animals and human volunteers, and epidemiologic information, suggest that immune protection against disease caused by *C. jejuni* is possible. However, the protective immune parameters, and the antigenic targets of protective immune responses, still remain to be defined fully.

Current Status of Norovirus Vaccine Development

ROBERT L. ATMAR, Departments of Medicine and Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS BCM 280, Houston, TX 77030, USA

Noroviruses are the most common cause of epidemic, non-bacterial gastroenteritis and are a common cause of foodborne disease. Human noroviruses are difficult to study because of the lack of available methods for *in vitro* cultivation and of small animal models. There are additional barriers to the development of effective norovirus vaccines, including the substantial antigenic variability seen among human noroviruses, the lack of cross-protection seen in limited studies of human experimental infection with heterologous strains, and the lack of well-defined correlates of protection from infection or disease. Nevertheless, evidence exists that protection from norovirus infection does occur, suggesting that development of an effective vaccine is possible. Current strategies have relied on the use of the major viral capsid protein, VP1, that, when expressed *in vitro*, forms viral-like particles (VLPs) that are antigenically and morphologically similar to native viral particles. When given orally, the VLPs induce both systemic (serum antibody) and mucosal immune responses in mice and in humans, and use of a mucosal adjuvant enhances these responses in mice. Intranasal VLP administration can induce more robust immune responses than the oral route. The immune response can be broadened by co-administration of VLPs derived from several strains. VLPs expressed in transgenic plants are also orally immunogenic. Efforts to develop human norovirus challenge pools are underway so that the protective efficacy of candidate vaccines can be evaluated in Phase II clinical trials. Continued progress in the understanding of basic norovirus biology is expected to aid efforts to develop an effective norovirus vaccine.

1:30 p.m. – 5:00 p.m.

S06 Symposium on Long-term Sequelae of Pathogens Transmitted by Food

Co-convenors: Indaue Mello-Hall and Marguerite A. Neill

Diarrhea is often considered the form of clinical illness most associated with foodborne transmission of infectious agents. However, some pathogens transmitted by food can cause non-diarrheal illness, and some infectious diarrheal illnesses can have long-term sequelae, often of a non-enteric nature. Recent assessments of the burden of foodborne disease in the United States have focused on acute illness to measure morbidity, mortality, and lost productivity. Less is known about the disease burden of post-infectious complications in defined populations. This symposium will provide an overview of long-term sequelae associated with recognized foodborne pathogens including *Campylobacter, Escherichia coli* O157:H7 and *Salmonella*. The possible association of foodborne infection with inflammatory bowel disease will be reviewed. For the most common bacterial foodborne pathogens, the economic costs of their long-term sequelae will be discussed and compared to the costs of acute illness. The aim of the symposium is to provide a more complete perspective on the burden of foodborne disease in order to improve analyses of prevention efforts.

1:30 The Gift That Keeps on Giving: Postinfectious Sequelae of Foodborne Pathogens — MARGUERITE A. NEILL, Brown Medical School and Division of Infectious Diseases, Memorial Hospital of Rhode Island, 111 Brewster St., Pawtucket, RI 02860, USA

1:45 Guillain-Barré Syndrome Associated with *Campylobacter jejuni* Infection — BAN MISHU ALLOS, Vanderbilt University School of Medicine, A-2200 Medical Center North, Nashville, TN 37232, USA

2:15 Incidence and Clinical Spectrum of Reactive Arthritis Following Foodborne Illness — JOHN M. TOWNES, Division of Infectious Diseases, Oregon Health & Science University, NRC-3, 3181 SW Sam Jackson Park Road, Portland, OR 97239-3098, USA

2:45 Break

3:15 Late Sequelae and Long-term Outcomes in Children with Shigatoxin-associated Hemolytic Uremic Syndrome — JOHN R. BRANDT, Pediatric Nephrology, Children's Hospital of New Mexico, UNMSOM Dept. PD MSC10-5590, 1 University of New Mexico, Albuquerque, NM 87131 USA

3:45 The Economic Costs of Long-term Sequelae of Selected Foodborne Pathogens — TANYA ROBERTS, Economic Research Service, US Dept. of Agriculture, 1800 M St., NW – Room S2079, Washington, D.C. 20036-5831, USA

4:15 Roundtable Discussion

5:00 Adjourn

The Gift That Keeps on Giving: Postinfectious Sequelae of Foodborne Pathogens

MARGUERITE A. NEILL, Brown Medical School and Division of Infectious Diseases, Memorial Hospital of Rhode Island, 111 Brewster St., Pawtucket, RI 02860, USA

To most persons, foodborne disease is primarily identified as a diarrheal illness, whose major manifestations are nausea, vomiting, and diarrhea, either with or without blood. Recognized complications are usually those that affect the gastrointestinal tract directly, such as gastritis (from vomiting), small bowel intussusception (from rotavirus), toxic megacolon, perforation, abscess, and death. Their close temporal proximity to the illness and their severity has facilitated their inclusion in assessments of the morbidity and mortality of foodborne disease. But some foodborne pathogens are associated with postinfectious sequelae that can develop weeks to months later, and in many instances, long after the causative agent has been eliminated from the gastrointestinal tract. Most postinfectious sequelae are extraintestinal. There is a dearth of reliable data on their occurrence because their true association with the antecedent gastrointestinal illness may not be recognized, and for most of these postinfectious complications, they are not reportable to public health authorities. This has hampered assessments of their contribution to the burden of foodborne infection. A reactive arthritis can be seen following several foodborne pathogens including *Salmonella*, *Campylobacter* and *Yersina*. Some cases of Guillain Barré syndrome, a demyelinating polyneuropathy, are triggered by an antecedent *Campylobacter* infection. The hemolytic uremic syndrome can follow the diarrheal phase of infection with *Escherichia coli* O157:H7 and a few other serotypes of Shiga-toxin producing *E. coli* (STEC). The pathophysiologic basis of many of these postinfectious sequelae is complex and not uniform across pathogens. Considerable work needs to be done to profile the frequency of occurrence, severity, and duration of postenteric sequelae in order to establish reasonably accurate estimates of their contribution to the overall burden of foodborne disease.

Guillain-Barré Syndrome Associated with *Campylobacter jejuni* Infection

BAN MISHU ALLOS, Vanderbilt University School of Medicine, A-2200 Medical Center North, Nashville, TN 37232, USA

Guillain-Barré syndrome (GBS) is a demyelinating disease of peripheral nerves and is one of the most common causes of acute flaccid paralysis in industrialized countries. Numerous studies have linked cases of GBS to infection with the foodborne bacteria, *Campylobacter jejuni*.

Three lines of evidence support an association between *C. jejuni* infection and GBS: anecdotal reports, serologic surveys, and cultural studies. Several investigators have succeeded in isolating *C. jejuni* from the stools of patients with GBS. Between 8–50% of GBS patients had *Campylobacter* cultured from their stools very soon after the onset of neurological symptoms. Because IgG and IgM antibodies to *Campylobacter* may remain elevated for up to six weeks, several studies of GBS patients were done in which patients' and controls' sera were assayed for antibodies to *Campylobacter*. Controlled serologic studies in North America, Europe, Asia, and Australia have demonstrated that GBS patients have a higher rate of preceding *Campylobacter* infections. Most studies showed that more than one-third of GBS patients had serologic evidence of antecedent *Campylobacter* infection. The mechanism of the association is thought to involve molecular mimicry: bacterial ganglioside-like epitopes mimic peripheral nerve components thereby triggering a cascade of inflammation. Infection with particular serotypes and genetic polymorphisms of *C. jejuni* may be more likely to trigger GBS.

In conclusion, *C. jejuni* is an important trigger of GBS. Patient and bacterial factors may increase the risk. Although these factors are not understood fully, it seems clear that particular strains of *C. jejuni* are more likely to be followed by GBS.

Incidence and Clinical Spectrum of Reactive Arthritis Following Foodborne Illness

JOHN M. TOWNES, Division of Infectious Diseases, Oregon Health & Science University, NRC-3, 3181 SW Sam Jackson Park Road, Portland, OR 97239-3098, USA

It is widely accepted that reactive arthritis (ReA) can complicate infections caused by foodborne pathogens such as *Salmonella*, *Campylobacter*, *Shigella*, and *Yersinia*. Estimates of how frequently this complication occurs are primarily based on outbreak investigations involving single bacterial clones in selected populations. Incidence estimates derived from such investigations may be skewed by the virulence properties of the infecting strain and the genetics of the outbreak population. Likewise, clinical descriptions of ReA are based primarily on outbreak investigations and clinic- or hospital-based studies that may be biased toward more severe disease. Few population-based studies of ReA have been done previously.

In this presentation, the current understanding of the epidemiology and clinical aspects of ReA will be reviewed briefly. The remainder of the presentation will focus on the findings of a recent population-based study of ReA that was sponsored by the FoodNet program of the Centers for Disease Control and Prevention. Incidence of and risk factors for rheumatologic symptoms and ReA following infection with *Salmonella*, *Campylobacter*, *Escherichia coli* O157, *Shigella*, and *Yersinia* were determined. Clinical findings in a subset of subjects will be described. Findings from this study will contribute to a better understanding of the clinical spectrum of illness resulting from foodborne pathogens, and will help improve estimates of the burden of illness from this complication.

Late Sequelae and Long-term Outcomes in Children with Shigatoxin-associated Hemolytic Uremic Syndrome

JOHN R. BRANDT, Pediatric Nephrology, Children's Hospital of New Mexico, UNMSOM Dept. PD MSC10-5590, 1 University of New Mexico, Albuquerque, NM 87131, USA

Hemolytic uremic syndrome (HUS) can be caused by ingestion of shigatoxin-producing *Escherichia coli*. As an increasing number of children have suffered and survived HUS, long-term sequelae of the disease have become increasingly evident. HUS is a multiorgan disease, which can lead to both acute and chronic organ injury. Over the last ten years, a growing body of evidence points to an ongoing risk of both renal and extrarenal sequelae after HUS. Children with severe HUS are at risk of multiorgan sequelae for which they may require lifelong surveillance. Even children with apparent complete recovery from HUS are at some risk of renal disease or insufficiency later in life. This presentation will review the pathophysiology of acute and chronic organ injury resulting from HUS and the current literature describing long-term outcomes in HUS survivors.

The Economic Costs of Long-term Sequelae of Selected Foodborne Pathogens

TANYA ROBERTS, Economic Research Service, US Dept. of Agriculture, 1800 M St., NW – Room S2079, Washington, D.C. 20036-5831, USA

In 1997, the Economic Research Service examined both acute human illnesses and selected sequelae for seven foodborne pathogens. The acute illness costs (including acute deaths) were estimated at $11.2 billion to $25.4 billion annually, and long-term sequelae costs were estimated at $8.4 billion to $11.7 billion, resulting in an estimate totaling $19.6 billion to $37.1 billion annually for the societal costs of human illnesses associated with these foodborne pathogens. Importantly, for *Escherichia coli* O157:H7 and *Toxoplasma gondii*, Buzby and Roberts found the societal costs of long-term sequelae exceeded the acute costs (illnesses and deaths). The long-term sequelae were valued with the "present value" method to incorpo-

rate the impact on health over the lifetime of people from the initial acute foodborne illness. Economists at the Food and Drug Administration, by including a different long-term sequelae arthritis, into their cost estimates for salmonellosis, have arrived at an annual figure of $28.1 billion for 9 foodborne pathogens. This paper will update the cost estimates for selected long-term sequelae using the most recent epidemiological estimates of cases. New economic methods for valuing costs of human foodborne illnesses on society will be examined and critiqued. Differences in the resulting estimates of the societal costs of long-term sequelae of foodborne illnesses will be compared and contrasted, including the impact of different methods of valuation used by economists.

8:30 a.m. – 12:00 p.m.

S10 Symposium on the Impact of Emerging Food Trends on Food Safety

Co-convenors: Jean E. Anderson, J. Stanley Bailey, and Theodora Morille-Hinds

Over recent years, the increased diversity of the population, along with changing consumer expectations for culinary variety, access to seasonal fresh foods year-round, and the desire for more simplistic, healthful food choices, has created new and unique challenges for food safety professionals. The symposium will begin with an overview of these emerging food trends from a consumer perspective. The remainder of the symposium will focus on the food safety implications of these trends with emphasis given to an increasing demand for natural ingredients and foods, the growth of organic offerings, probiotics, preferences for diverse, exotic, fresh foods, and the issues and strategies they have prompted within the food industry. The impact of food manufacturing using minimal and non-traditional processing methods to meet consumer expectations will also be examined.

8:30 Emerging Consumer Trends: Challenges for Food Safety — CHRISTINE M. BRUHN, Center for Consumer Research, Dept. of Food Science and Technology, University of California-Davis, One Shields Ave., Davis, CA 95616-8598, USA

9:00 Safety of Probiotics — PIETER BREEUWER and A. Constable, Nestlé Product Technology Centre Konolfingen, Nestlé Strasse 3, CH-3510 Konolfingen, Switzerland

9:30 Natural Ingredients and Food Trends — JOSEPH D. MEYER, Scientific Affairs & Regulatory Affairs, Kraft Foods Global, Inc., 910 Mayer Ave., Madison, WI 53704, USA

10:00 Break

10:30 Organic Foods and Food Safety: Separate, Antagonistic, or Symbiotic? — DOUGLAS A. POWELL, Katija Morley, Stacey Cahill, Benjamin C. Chapman and Amy L. Hubbell, International Food Safety Network, Kansas State University, Manhattan, KS 66506, USA

11:00 Food Safety Challenges Posed by Minimal/Opinion-traditional Food Processing Technologies — MARTIN B. COLE, National Center for Food Safety and Technology, 6502 South Archer Road, Summit-Argo, IL 60501, USA

11:30 Food Safety Challenges and Strategies Allowing for Unique, Year-round, Globally-sourced Ingredients, Commodities, and New Products — SARA E. MORTIMORE, Global Sourcing Development, General Mills, Inc., Number One General Mills Blvd., WO4B, Minneapolis, MN 55426, USA

12:00 Adjourn

Emerging Consumer Trends: Challenges for Food Safety

CHRISTINE M. BRUHN, Center for Consumer Research, Dept. of Food Science and Technology, University of California-Davis, One Shields Ave., Davis, CA 95616-8598, USA.

Consumer demands for convenience, quality, and novelty impose additional challenges in food safety. Convenience isn't a luxury today; it is essential. Time savings alone is not sufficient. Consumers want foods that are fast to prepare, with the flavor qualities of freshly prepared, and a nutrient profile that will correct all the problems of a population that is aging, less active, and not inclined to follow all the dietary guidelines. In the supermarket, consumers are turning more to time-saving fresh dishes, and choosing frozen less often. People want entrees, fruits, vegetables, and snacks, with preparation steps done by others. At the same time, many seek products without additives, preservatives or processing innovations that could protect safety. Successful new products often feature foods with new flavor combinations. Novel flavors and the quest for low ingredient or production costs may lead to increased sourcing of foods outside the United States. This may bring unexpected safety problems even in processed products. The impact of an error in handling is multiplied when this error occurs in the food service sector because people are increasing their reliance on supermarkets, fast food, and restaurants to prepare meals fully. The demographic profile of consumers in the United States is changing as well, with more people at increased risk for foodborne disease because of weakened immune systems as the result of health conditions such as diabetes, treatment of medical conditions, or living to ages older than 55 or 60 years. These factors combine to create an environment with high public expectations and high food safety vulnerability.

Safety of Probiotics

PIETER BREEUWER and A. Constable, Nestlé Product Technology Centre Konolfingen, Nestlé Strasse 3, CH-3510 Konolfingen, Switzerland

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. In the last decade, the consumption of probiotics, either directly or via addition to food products, has become more and more popular. Most probiotics are bacteria from well known genera such as lactic acid bacteria, traditionally used as starter cultures in the dairy industry in, e.g., yogurt and cheese production, and have a long history of safe use. Nevertheless, the increased importance of probiotics has also led to a more in-depth analysis of the safety status of these ingredients. For instance:

1. With the increased demand for health benefits, new microorganisms are now explored for their probiotic potential. For most of those uncommon strains there is a need to obtain more information about their safety.
2. There has been a growing understanding that commensal bacteria may represent a reservoir of antibiotic resistance traits, that have the potential to be transferred to pathogenic bacteria. In this respect, there is a concern that starter cultures and probiotics may contain antibiotic resistance determinants.
3. The production of probiotics on a large scale is highly specialized and demands strong expertise in Good Manufacturing Practices and adequate analytical methods for analysis of contaminants.
4. Probiotics in current use are considered safe for the general population. Only in some cases, where the consumer heath status is severely compromised, should probiotics be administered with caution. This presentation will discuss these points in detail.

Natural Ingredients and Food Trends

JOSEPH D. MEYER, Scientific Affairs & Regulatory Affairs, Kraft Foods Global, Inc., 910 Mayer Ave., Madison, WI 53704, USA

Consumers today are continuing to explore a variety of options when making food purchases. The area of natural and less processed foods continues to be of interest to consumers looking for different food choices. Ingredient options can be limited for these foods, and it can be a challenge for food manufacturers to deliver on the consumers' desire for natural ingredients while meeting their expectation of minimal spoilage and minimal safety risk.

In recent years there has been a tremendous research effort to identifying natural ingredients that could be used to inhibit spoilage or pathogenic microorganisms in food products. Many of the potential ingredients identified have failed to be utilized successfully in food products for a variety of reasons. However, there are products in the market place that incorporate natural ingredients and other strategies to deliver natural products to consumers that will also meet their expectations of minimal spoilage and minimal safety risk from microbial growth. Several examples will be presented with a comparison to their conventional food counterparts.

Organic Foods and Food Safety: Separate, Antagonistic, or Symbiotic?

DOUGLAS A. POWELL, Katija Morley, Stacey Cahill, Benjamin C. Chapman, and Amy L. Hubbell, International Food Safety Network, Kansas State University, Manhattan, KS 66506, USA

Fresh fruits and vegetables have been identified as a significant source of microbial foodborne illness for at least the past decade. Outbreaks have been linked to both conventionally and organically grown produce.

Previous studies have identified gaps between US Food and Drug Administration on-farm food safety guidelines and the US Department of Agriculture's organic standards in terms of microbial food safety. Although microbial food safety standards are often achieved indirectly under organic production, organic standards are process-based, and have nothing do to with end-product safety. Specific omissions include worker hygiene and recommendations for safe use of processing and irrigation water. Further, any guideline or standard is meaningless without robust verification. The production of safe food is the responsibility of everyone in the farm-to-fork chain — conventional or organic — and food safety, especially with fresh produce, must begin on the farm.

Food Safety Challenges Posed by Minimal/ Opinion-traditional Food Processing Technologies

MARTIN B. COLE, National Center for Food Safety and Technology, 6502 South Archer Road, Summit-Argo, IL 60501, USA

The rapid globalization of the food processing and retailing industries, consumer demand for more natural and more convenient products, and an overall increase in the susceptibility of the population are believed to be the most important factors that have led to fundamental changes in the nature of food-borne disease. The primary consumer trends within the mature food markets have been toward products perceived as offering pleasure, health, and convenience benefits. Within this environment, the food industry has been plagued by on-going food safety issues and dietary concerns that include bacterial pathogens, bovine spongiform encephalopathies (BSE), chemical contaminants, allergens, and obesity. Food safety is a recognized given in the market place yet consumer trends towards fresher, more natural, less preserved, convenient foods are not always conducive to enhanced food safety as many traditional preservation regimes are also intrinsic stability or food safety factors. Recent advances in food science and technology, such as risk assessment, genomics, and novel preservation, offer exciting new possibilities for innovation to meet the consumer drivers of health, convenience, pleasure, and environment. In delivering these possibilities, it is important that we do not introduce new food safety hazards. This will require

not only the use of new technologies, but also an intricate networking and collaboration among all stakeholders involved. This paper will review developments in minimal and non-traditional food processing technologies, the opportunities these technologies offer for innovation, and steps that are being taken to minimize the food safety challenges from their adoption.

Food Safety Challenges and Strategies Allowing for Unique, Year-round, Globally-sourced Ingredients, Commodities, and New Products

SARA E. MORTIMORE, Global Sourcing Development, General Mills, Inc., Number One General Mills Blvd., WO4B, Minneapolis, MN 55426, USA

Global sourcing of food, while not new, is increasing as consumers demand year-round produce and innovative products, and manufacturers seek low-cost opportunities. Global food trade requires global food safety standards as well as compliance with the importing countries' regulatory requirements. Challenges are many and varied, meaning that there is no one single risk mitigation strategy – flexibility is key.

Many food producers, particularly in the developing countries, do not yet comply fully with the basic food manufacturing practices of a hygienic operating environment and equipment designed to high sanitary standards. Cross-contamination prevention, pest control, allergen management, storage at safe temperatures, and sanitation programs are often inadequate. That, combined with poor understanding of HACCP and microbiology, leads to a high likelihood of failure.

TECHNICAL ABSTRACTS

T1-01 Ten-minute Assay for Detecting *Escherichia coli* O157:H7 in Ground Beef Samples Using Piezoelectric-excited Millimeter-sized Cantilever Sensors

David Maraldo and RAJ MUTHARASAN, Drexel University, 3141 Chestnut St., Philadelphia, PA 19104, USA

Introduction: Detection of foodborne pathogens involves sample collection, and enrichment, followed by selection and identification of the targeted organism by use of labeled reagents. When the pathogen is present at a very low concentration, time-to-result (TTR) could take 8–24 h.

Purpose: The purpose is to establish feasibility and determine detection limit of cantilever sensors for detecting *E. coli* O157:H7 (EC) in ground beef samples.

Methods: Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors were fabricated and immobilized with polyclonal antibody (Ab) specific to EC. Ground beef (2.5 g) in 10 ml phosphate buffered saline was spiked with EC at 10–10,000 cells/ml. One ml of supernatant was removed from each blended sample and used in detection experiments.

Results: The PEMC sensor's resonance frequency decreases when target EC binds to it. The total resonant frequency change obtained for the samples was 138 ± 9, 735 ± 23, 2,603 ± 51, and 7,184 ± 606 Hz, corresponding to EC concentrations of 3, 25, 250, and 2,500 cells/ml, respectively. Positive detection of EC in the sample solution was observed within 10 min. The response to control samples was negligible i.e., Positive (EC present, no Ab), negative (EC absent, Ab), and buffer (EC absent, no Ab) controls gave 36 ± 6, 27 ± 2, and 2 ± 7 Hz, respectively. Positive verification of EC attachment was confirmed by low-pH buffer (PBS/HCl pH 2.2) release, microscopic analysis, and second antibody binding post-EC binding. The results indicate that PEMC sensors can reliably detect *E. coli* O157:H7 at less than 10 cells/ml in 10 min without sample preparation, and with label-free reagents.

Significance: The 10-minute TTR method developed in this study with beef samples is applicable for detecting any pathogen in other food matrices with the appropriate antibody immobilized on the sensor.

T1-02 Rapid Detection of *Listeria monocytogenes* Using Quantum Dots and Nanobeads-based Optical Biosensor

HONG WANG, Y. Li, and M. Slavik, University of Arkansas, O-114 Poultry Science Bldg., Fayetteville, AR 72701, USA

Listeria monocytogenes is one of the major foodborne pathogens associated with poultry and meat products. Current conventional detection methods are still limited because of their time-consuming processes, lack of specificity or quantitative results, complex procedures, and high cost.

The objective of this study was to develop a rapid, sensitive and selective method for quantitative detection of *L. monocytogenes* in poultry and meat products by use of magnetic nanobeads to separate and concentrate the target bacteria, and quantum dots as fluorescent markers in an optical biosensor. Both streptavidin conjugated quantum dots (605 nm emission wavelength) and magnetic nanobeads (140 nm diameter) were separately coated with biotin-conjugated anti-*L. monocytogenes* antibodies. The conjugated magnetic nanobeads then were mixed with a poultry or meat sample to capture, separate and concentrate *L. monocytogenes* that was inoculated into the sample. After immunomagnetic separation, the magnetic nano *L. monocytogenes* conjugates were mixed with the conjugated quantum dots. Unattached conjugated quantum dots were removed, using immunomagnetic separation. A fluorescence spectrometer was used to measure fluorescence intensity of the complexes of nanobeads–*L. monocytogenes* quantum dots. Results indicated that this method could detect *L. monocytogenes* at a concentration as low as 5–10 CFU/ml in a pure culture or a food sample (chicken carcass washwater or ground beef).

A linear relationship was found between the fluorescence intensity and *L. monocytogenes* concentration in a range of 10^0 to 10^7 CFU/ml. The total detection time including sampling and measurement was less than 1.5 h. The results also showed that this method has potential to be able to detect a single cell of *Listeria* when the sample is incubated for 1 to 2 h prior to detection. This quantum dots and nanobead-based biosensing method may provide a better alternative way to detect *Listeria monocytogenes* rapidly, sensitively and quantitatively in poultry and meat samples for ensuring food safety.

T1-03 Methods for the Detection of Foodborne Viruses: Approach to Quantification

FABIENNE LOISY-HAMON, Soizick Le Guyader, and Benoit Lebeau, CEERAM SAS, 22 Ave. de la Passagère, Saint Herblain, 44800, France

Introduction: Foodborne viral infections are a common source of gastrointestinal disease. The proportion of outbreaks attributed to viral agents, predominantly norovirus, has increased regularly every year. In December 2006, an increase in noroviruses activity was reported to European national health authorities via the Foodborne Viruses in Europe network.

Purpose: Based on a proposal of the CEN (European Committee for Standardization) working group on virus detection in foods, standardized methods were evaluated for the detection of noroviruses (genogroup 1 and 2) and hepatitis A virus in food (shellfish, fruits, vegetables and bottled water).

Methods: Following virus concentration, a semi-automatic method based on the Boom protocol using magnetic silica was evaluated. This method allowed the extraction and purification of 12 samples in 4 h, whatever the analyzed matrices. An extraction control was systematically used to validate the method and to determine the extraction efficiency. One step real time RT-PCR were developed in conserved region of the genome for each virus to reach a sensitivity of 5 genome copies at a confidence level superior to 95%. Internal controls and standards were developed to monitor inhibition and quantification.

Results: For shellfish collected in recreational areas during one year, a contamination level ranging from 30 to 5,105 genome copies/shellfish was detected for noroviruses. In market shellfish, most of the samples were negative, but one was found positive, with a contamination of 3,104 genome copies/shellfish for norovirus genogroup 2. Analyses were also performed on raspberries implicated in an outbreak; the contamination was 3,104 genome copies/25 g of fruits for norovirus genogroup 2.

Significance: These improved methods allow for rapid detection, identification and quantification of foodborne viruses for different types of food in 24 to 48 h, depending on the urgency.

T1-04 Development and Application of Real-time NASBA for the Detection of Norovirus in Lettuce Samples

Safaa Lamhoujeb, Solange E. Ngazoa, Ismaïl Fliss, and JULIE JEAN, Universite Laval, Dept. Food Science and Nutrition, Comtois Bldg. Room 1401, Quebec, QC, G1K 7P4, Canada

Norovirus is a significant worldwide public health concern causing viral gastroenteritis. In the absence of culture methods, detection of noroviruses in foods relies on molecular techniques based on viral RNA amplification and amplicon detection by hybridization and/or sequencing. Furthermore, the high genetic diversity that exists among noroviruses requires a specific method able to detect all norovirus genetic variants. Among new proposed molecular techniques, NASBA has shown great efficacy for direct amplification of viral RNA. In this study, NASBA primers and molecular beacon were designed in the most conserved region of norovirus genome, the ORF1-ORF2 junction, to develop a real-time, sensitive and specific detection method for genogroup II norovirus. Results were compared to those obtained by TaqMan RT-PCR and then validated on spiked lettuce samples. Using optimized conditions, 0.01 RT-PCR detectable units of norovirus was achieved. This sensitivity is comparable to that obtained by the TaqMan real-time RT-PCR. Specificity of the real-time NASBA assay was evaluated on 96 clinical samples isolated from different Canadian provinces, with 89% of the samples confirmed as norovirus positive by NASBA, compared to 86% by TaqMan RT-PCR. Similar specificity was obtained with spiked lettuce samples, indicating the suitability of the assay for detection of viral contamination in foods.

T1-05 Detection of Avian Influenza Virus H5N1 Using an Impedance Biosensor-based on Immuno-nanobeads and Microfluidic Biochips

YANBIN LI, Ronghui Wang, Billy Hargis, Steve Tung, and Luc Berghman, University of Arkansas, 230 Engineering Hall, Fayetteville, AR 72701, USA

A key to the control of avian influenza (AI) is rapid detection of the disease followed by quarantine and vaccination measures. Current methods used to detect avian influenza, such as viral culture, RT-PCR and ELISA, are either time consuming, too expensive, or not specific to subtypes of AI viruses, or, most importantly, they require a laboratory and a highly trained technician. Therefore, the objective of this research was to develop an impedance biosensor to detect AI virus H5N1 in the field rapidly. An impedance biosensor was designed based on three novel concepts: (1) magnetic bio-nanobeads for efficient and rapid separation of target virus in a sample; (2) a microfluidic biochip with interdigitated array microelectrode for delivery, capture and measurement of target virus; and (3) the complexes of red blood cells and nanobeads for amplification of impedance signal. Magnetic nanobeads (140 nm diameter) were coated with anti-H5 antibodies and used to separate and concentrate AI virus H5N1 in a sample. In the biochip, a microfluidic channel (40 μm depth and 100 μm width) and an interdigitated array microelectrode (25 pairs of electrode fingers with 15 μm width) coated with specific antibodies were designed for impedance measurement of target virus. The results showed that the biosensor was able to specifically detect inactivated AI virus H5N1 at a concentration as low as 100 EID50/ml in a poultry cloacal swab sample in less than 30 min. This is more rapid and more sensitive than any other currently available rapid detection method. The capture of target AI virus by use of immunonanobeads was more efficient than with use of centrifuge, filtration and microbeads. Other species of viruses in the swab sample did not interfere with the specific detection of AI virus H5N1. This study may lead to a portable instrument for quantitative detection of multiple subtypes of avian influenza viruses, which would make in-field detection of AI possible.

T1-06 DSC Enumeration of Viable Cells of *Listeria monocytogenes* in Biofilms by Use of Propidium Monoazide and Real-time PCR

YOUWEN PAN and F. Breidt, Jr., USDA-ARS and North Carolina Agricultural Research Service, Dept. of Microbiology, North Carolina State University, 322 Schaub Hall, Campus Box 7624, Raleigh, NC 27695, USA

Both ethidium monoazide (EMA) and propidium monoazide (PMA) have been reported to be used for cross linking with DNA from dead cells of microorganisms, allowing live bacteria only to be enumerated in a live/dead mixture by PCR detection methods. The purpose of this study is to compare the two fluorescent dyes and select one for live cell quantification in *Listeria monocytogenes* biofilms. EMA was found to kill the bacterium during incubation and light exposure,

presumably by intercalating into DNA of live cells. However, PMA had no apparent affect on the growth or survival of *L. monocytogenes*. The amount of DNA that could be amplified by real-time PCR from live cells treated with EMA was significantly less than that from non-EMA treated cells. Live *Listeria* was subjected to a range of temperatures prior to EMA treatment. Increasing temperatures resulted in a decrease in the amplification of DNA from EMA treated live cells. Amplification of DNA from live cells was not inhibited by PMA regardless of temperature. In quantification of live cells of *Listeria* in biofilms by real-time PCR, PMA was found to prevent amplification of DNA from dead cells as well as or even better than EMA under optimal conditions. For real-time PCR for quantification of viable cells of *Listeria*, PMA was more reliable than EMA under a variety of conditions, including in biofilms. The PMA-PCR assay potentially provides significant applications in food safety research.

T1-07 DSC Evaluation of Loop-mediated Isothermal Amplification to Detect *Vibrio vulnificus*

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Introduction: According to the most recent CDC FoodNet report, alarmingly, human infections caused by *Vibrio vulnificus* due to eating raw or undercooked oysters have shown a sustained increase. Simple, rapid, and sensitive detection methods are urgently needed to better ensure oyster safety. The recently invented loop-mediated isothermal amplification method (LAMP) provides a promising alternative for detecting foodborne pathogens in foods and has been applied in detection of a handful of microorganisms to date.

Purpose: The purpose of this study was to evaluate the LAMP technique for rapid and sensitive detection of *V. vulnificus*.

Methods: A set of four primers, two outer and two inner, was designed specifically to recognize the vvhA gene of *V. vulnificus*. The LAMP reaction mix was optimized by varying the concentrations of $MgSO_4$, betaine, deoxynucleotide triphosphate (dNTP), enzyme, inner and outer primer ratio, and assay temperature and duration.

Results: The optimum amplification result could be obtained using 6 mM of $MgSO_4$, 0.8 M of betaine, 1.2 mM of dNTP, 1:10 of the inner and outer primer ratio, at 65°C for 1 h. Those conditions could effectively amplify the ladder-like banding pattern in sizes of 204 bp and above specifically for the *V. vulnificus* vvhA gene. In addition, the detection limit of this LAMP assay was determined to be ca. 30 colony forming units in pure culture.

Significance: The results suggest that the LAMP technique may be adopted for rapid and sensitive detection of *V. vulnificus*.

T1-08 Comparison of Methods for Detecting Live, Stressed, and Dead Cells of *Campylobacter jejuni*

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Introduction: The microaerophilic gram-negative bacterium *Campylobacter jejuni* is the leading cause of foodborne illnesses. It can be found in high prevalence in poultry products and in many environments, yet it is difficult to culture in the laboratory. When stressed, it can assume a "viable but non-culturable" (VBNC) state, with a concomitant change in morphology from a spiral to a coccoid-shape.

Purpose: The purpose of this study was to compare several methods for detecting and/or quantifying live/viable and dead bacteria to evaluate cell viability under different growth and stress conditions.

Methods: Ethidium monoazide in combination with real-time PCR (EMA-PCR), BacLight viability staining with confocal microscopy, and plate counting on Mueller-Hinton (MH) agar were used to quantify cells in different growth stages and under stress conditions such as exposure to oxygen (in air/1 week), heat (boiling/ 10 min), and isopropanol (30 min). *Campylobacter jejuni* strain 81–176 was cultured in MH broth or on MH agar under microaerobic conditions (5% oxygen, 10% carbon dioxide, 85% nitrogen) for 16 h prior to stress treatments or before dilution for growth studies.

Results: Plate counting, microscopy and EMA-PCR showed varying degrees of detection sensitivity for live, dead, and stressed (coccoid) cells. The plate count failed to detect culturable cells under oxygen-stressed conditions even when both EMA-PCR and BacLight staining showed that the majority of the cell population (> 96%) was viable. The EMA-PCR method, though sensitive, could not differentiate the cell populations containing more than 99.9% dead cells and thus may overestimate the number of viable cells. Microscopy with viable-staining is labor-intensive and not appropraite for routine laboratory testing.

Significance: The ability to detect viable bacteria is critical in evaluating the efficacy of intervention strategies and for risk assessment. Conventional culturing methods could underestimate the bacterial counts, especially for cells in the VBNC stage, resulting in "fail-danger status" in intervention processes.

T1-09 Evaluation of the VIDAS® *Listeria* Species Xpress with Ottaviani Agosti Agar Method for the Detection of *Listeria* Species in Poultry, Seafood, and Vegetable Products: AOAC Food Matrix Extension Study

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The objective of this study was to extend the food matrices validated for use with the VIDAS® *Listeria* species Xpress (LSX) method, which includes a 30-h proprietary enrichment broth, enabling next-day release of test samples that are screened negative for *Listeria* by the VIDAS LSX test. The VIDAS LSX has been previously validated with Ottaviani Agosti chromogenic media as an AOAC Performance Tested Method SM (PTM) for use with meat products and dairy products and 8 environmental surfaces. A food matrix extension study was conducted to validate the VIDAS LSX method for the detection of *Listeria* species in artificially contaminated foods including

poultry, vegetables and seafood products. In this food matrix extension study, seven food types—chicken franks, fresh ground chicken, pasteurized crab meat, cod fillets, raw shrimp, green beans and cauliflower—inoculated at fractional positive levels were analyzed by the VIDAS LSX method and the appropriate reference methods, FDA Bacteriological Analytical Manual (BAM) or USDA/FSIS Microbiology Laboratory Guidebook (MLG). The LSX method was significantly more sensitive ($x^2 = 7.7$) than the FDA/BAM Method for the detection of *L. grayi* in pasteurized crabmeat, and OAA was significantly more sensitive than OXA in confirming *L. grayi* from pasteurized crabmeat. For all other foods tested in the method extension study, no significant difference ($x^2 < 3.84$) was observed between the performance of the alternative method and the combined standard methods. The LSX method demonstrated 100% specificity, with no false positive results. With the AOAC PTM matrix extension approval, this method has been validated as a next day screen for the presence of *Listeria* species in a variety of foods, including vegetables, seafood, poultry, meat and dairy products.

T1-10 DSC Rapid Enrichment and Detection of *Salmonella* in Composite Raw Beef Samples: A Comparison between Cultural Method, PCR and Lateral Flow Immunoassay

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A successful food safety program requires a rigorous sampling and testing plan. A general trend in the food industry is the use of composite samples to increase sensitivity and to reduce the cost of food pathogen testing. For example, most labs test for *Escherchia coli* O157 with 375 g composite samples, instead of 25 g or 65 g, a practice that has been widely accepted. Compositing plans for *Salmonella* testing are still needed. In this work, *Salmonella* enrichment and detection in raw ground beef trim product was tested, at composite size of 125 g per sample with 1125 ml of primary media was added to each sample. This was compared to the USDA cultural method, automated PCR method and a lateral flow assay (LFA) method. A total of 108 samples were processed, of which 38, 32 and 38 confirmed positives were correctly reported for the three methods, respectively. The sensitivity and specificity are 82% and 100% for the PCR method, and 100% and 93% for the LFA method, respectively. A total of 79 natural *Salmonella* isolates have been obtained, and at least 50% of them associated with the C1 group. Apparently due to the low productivity of buffered peptone water used in the PCR method as primary enrichment, a significant high false negative rate (18%) was reported with this method for composite samples compared to reference. This work indicates that proprietary enrichment followed by the lateral flow assay provides a reliable alternative test method for *Salmonella* in composite beef samples.

T1-11 Design of a Novel Multiplex Real-time PCR Assay for *Vibrio* Pathogen Detection, Quantification and Speciation

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Introduction: The presence of pathogenic organisms in our food and environment is a constant threat to human health. FDA and USDA regulations require that our nation's food supplies be tested for the presence of common foodborne pathogens. Classical methods can require up to one week for confirmatory results as to the presence of a pathogenic organism.

Purpose: The purpose of this study was to develop a real-time PCR assay that would shorten time to result for detecting multiple *Vibrio* species in a single assay.

Methods: A multiplex real-time PCR assay was designed to identify 3 species of *Vibrio* and an internal positive control by use of a four-dye configuration. Because of sequence diversity, multiple strains were sequenced within each species to identify target sites, and several targets were identified for each species to account for assay incompatibilities during multiplexing. The sensitivity of the assay and efficiency for quantifying pathogen levels were determined, using *Vibrio* genomic DNA and a mixture of pathogens.

Results: *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were detected either individually or in mixtures with a sensitivity of ≤ 25 genomic copies. The *Vibrio* multiplex assay showed 100% specificity to all targets analyzed (51/51 *V. cholerae*, 19/19 *V. parahaemolyticus*, and 14/14 *V. vulnificus* tested positive), and no detection of an exclusion panel (30 *Vibrio* strains representing 9 different *Vibrio* species, including nearest neighbors). Each assay in the multiplex exhibited 100 ± 10% efficiency over a 5 log range of target concentrations.

Significance: Multiplex real-time PCR can simplify food testing and significantly reduce costs since 3 species can be analyzed in a single reaction tube. Multiplexed PCR assays can be designed for any set of pathogens to provide detection and define pathogen load.

T1-12 Five-hour Real-time PCR-based Method for Rapid and Simultaneous Detection of Five Foodborne Pathogenic Bacteria in Food

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A new real-time PCR assay was successfully developed using a SYBR Green I for specific detection and enumeration of five pathogenic bacteria, *E. coli* O157:H7, *S. aureus*, *V. parahaemolyticus*, *L. monocytogenes* and *Salmonella* spp., in food. The specific primers for multiplex PCR amplification of Shiga-like toxin (verotoxin Type II), femA (cytoplasmic protein), toxR (transmembrane DNA binding protein), iap (invasive associative protein), and invA (invasion protein A)

gene sequences. The minimum detection limit of this technique was approximately 1 CFU/ml of a pure culture. The assay was then applied to milk and pork in multiplex different trails. The cell population detected 10 CFU/g of the strains without an enrichment procedure. The ability to test for the five bacteria simultaneously will save time and increase the ability to assure food quality.

T2-01 Survival and Transport of *Escherichia coli* O157:H7 in Agricultural Environments

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Escherichia coli O157:H7 has been shown to contaminate fresh produce. This pathogen can survive in low numbers in water, soil and on plants. Nevertheless, pre-harvest contamination likely occurs by application of raw or poorly composted manure to the crop or inadvertently from livestock or wild animals. Several recent outbreaks associated with lettuce and spinach have focused attention on Salinas and the surrounding region. Nine hundred sixty-four *E. coli* O157:H7 isolates were recovered from the Salinas watershed. MultiLocus Variable number tandem repeat Analysis (MLVA) was used for high-throughput typing, yielding 57 different types. Phylogenetic analysis indicated that related *E. coli* O157:H7 were isolated generally in the same proximity. Isolates from distant portions of the watershed were less related MLVA types. Furthermore, in one region of the watershed a point source of *E. coli* O157:H7 contamination was discovered. During a low stream flow situation, closely related pathogens were detectable at 10, 60 and 139 meters downstream from the source, but were undetectable at 229 meters and further downstream. Beyond 139 meters, the pathogen may loose viability or, more likely, become diluted to undetectable levels. Nevertheless, from all the watershed, a few strains with identical MLVA types were collected on the same day at points separated up to 24 kilometers apart. However, unlike the previous example, these isolates were collected exclusively during flooding conditions and indicate that the pathogen may be transported long distances under certain conditions. During the baby spinach outbreak investigation in October 2006, hundreds of samples were taken in or near 2 implicated farms and yielded more than 30 MLVA types. These types were again clustered spatially. Furthermore, pathogens identical to the outbreak strain were isolated from several sample types (including cattle, surface water, sediment, dust and feral pig), but only within a few miles of one implicated field. Pre-harvest contamination of spinach may have occurred by transport of the pathogen from the surrounding environment to the fields by feral pig or dust. These data suggest that MLVA is an effective high thoughput investigation tool of the transport of *E. coli* O157:H7 in agricultural environments, especially following pre-harvest contamination events.

T2-02 Efficacy of Hot Water Surface Pasteurization vs. Chlorine and Experimental Sanitizing Wash Treatments for Reducing Populations of *Salmonella* Poona on Inoculated Whole Cantaloupe Melons

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Numerous outbreaks of salmonellosis by *Salmonella* Poona have been associated with the consumption of cantaloupes. Commercial washing processes for cantaloupes are limited in their ability to inactivate and/or remove this human pathogen. The objective of this study was to compare the efficacy of hot water surface pasteurization at 76°C for 3 min to that of various chlorine and experimental sanitizer solutions for the inactivation of *Salmonella* on inoculated cantaloupe surfaces. Whole cantaloupes, surface inoculated with S. Poona RM 2350 to a final cell concentration of 5–6 log CFU/cm^2, were stored at 4°C or room temperature (RT = 19°C) for up to 48 h prior to processing. Washing treatments with tap water at 76°C for 3 min at 24 and 48 h post inoculation resulted in excess of 5 and 3 log CFU/cm^2 reductions in S. Poona and in yeast and mold populations, respectively. All washing treatments with aqueous sanitizers at RT for 20 min resulted in less than 2 log reductions in S. Poona and in yeast and mold populations. These results demonstrate the utility of hot water for the inactivation of *Salmonella* on cantaloupes and provide a framework to producers of fresh-cut melon for the potential use of hot water as an intervention treatment for enhancing the microbiological safety and extending the shelf life of this commodity. Storage of untreated inoculated cantaloupes at RT for up to 48 h post inoculation caused a significant ($P < 0.05$) increase in S. Poona as compared to storage at 4°C. This indicated that cantaloupes should be refrigerated as soon as possible following harvesting to suppress the growth of any possible contaminant on the rind. These findings will assist food industry and regulatory agencies in establishing processing guidelines to guard against pathogens, thereby decreasing the incidence of food poisoning outbreaks.

T2-03 Reduction of *Escherichia coli* O157:H7 and *Salmonella* Species on Baby Spinach by Use of Electron Beam Irradiation

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Introduction: A recent *E. coli* O157:H7 out-break has researchers studying ways to improve the safety of spinach. Despite the use of proper hygiene and Good Agricultural Practices, which are the most effective means of obtaining safe produce, contamination may still occur. Aqueous sanitizers may not be sufficient, warranting the use of other methods. One possible method may be the use of electron beam irradiation.

Purpose: The purpose of this study was to test the reduction of inoculated *E. coli* O157:H7 and *Salmonella* on baby spinach.

Methods: Fresh spinach was inoculated with a bacterial cocktail containing rifampicin resistant (Rif+) *E. coli* O157:H7 and Rif+ *Salmonella* Agona, Gaminara, Michigan, Montevideo, Poona and Typhimurium. Inoculated samples as well as controls were exposed to 0.79, 1.16 or 2.48 kGy electron beam irradiation from a linear accelerator. Reductions in populations were determined by plate counting. Irradiated spinach was also stored for 8 days at 4°C and counting was done at 2-day intervals to determine if there was any effect of irradiation on the survival of both pathogens.

Results: For *E. coli* O157:H7, the log reduction was 4.1, 6.3 and 6.4 log CFU/g when irradiated at 0.79, 1.16 and 2.48 kGy, respectively. For *Salmonella*, the log reduction was 4.0 at 0.79 kGy. For samples irradiated at 1.16 or 2.48 kGy, initial counts of 7.3 log CFU/g were reduced to below the detection limits (< 10 CFU/g). Irradiation did not affect the survival of the remaining microorganisms stored over eight days ($P < 0.05$).

Significance: These results suggest that electron beam irradiation may be a viable tool for reducing microbial populations or eliminating *E. coli* O157:H7 and *Salmonella* from fresh bagged spinach.

T2-04 DSC Decontamination of Fresh Cut Produce by Using a Combination of Ultraviolet Light and Hydrogen Peroxide

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Introduction: Over the last decade there have been over 34 foodborne illness outbreaks linked to minimally processed vegetables (e.g., lettuce, spinach) contaminated with pathogens such as *Escherichia coli* O157:H7 and *Salmonella*. The microbiological safety of fresh produce is compromised by the absence of an effective intervention step to inactivate field acquired contamination. This is partly due to the limited efficacy of sanitizer based washes to inactivate pathogens located within the sub-surface tissue of produce.

Purpose: A produce decontamination method has been developed based on using a combination of UV and hydrogen peroxide. When hydrogen peroxide is activated by UV photons, short lived but highly reactive antimicrobial radicals are formed that can potentially inactivate pathogens within sub-surface structures.

Methods: The treatment chamber consisted of a low pressure 12W UV (254 nm) lamp. Hydrogen peroxide was held within a reservoir and delivered (480 ml/min) as a fine mist via passage though 3 screen filtered nozzle heads with an aperture of 1 mm Iceberg lettuce was inoculated by using submersion in bacterial suspensions (*Salmonella* or *E. coli* O157:H7) followed by vacuum infiltration to internalize pathogens.

Results: Optimum log reductions were achieved by delivering the hydrogen peroxide spray (1.5% v/v at 50°C) for 20 s followed by a UV exposure for a further 30 s. Surface counts of *Salmonella* and *E. coli* O157:H7 on lettuce were reduced by 4.12 and 3.87 log CFU respectively. Log reductions in internal counts were 0.8–1.2. In comparison, hypochlorite (200 ppm) reduced surface counts by 0.5 log, and no decrease in internal pathogen populations occurred. No significant changes in the visual appearance, or post-treatment recovery of pathogens, occurred with treated lettuce stored for 8 days at 4°C.

Significance: UV:hydrogen peroxide based treatment provides an effective alternative to sanitizer-based washes for decontamination of fresh produce.

T2-05 Inactivation of *Escherichia coli* O157:H7 Internalized in Leaves of Romaine Lettuce and Baby Spinach: Sodium Hypochlorite Wash vs. Irradiation

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Pathogenic bacteria that are internalized in leaf tissues are protected from the antimicrobial effects of surface treatments. Ionizing radiation is known to penetrate foods, but the efficacy of the process against internalized bacteria is unknown. Leaves of romaine lettuce and baby spinach were cut into pieces, submerged in a cocktail mixture of three isolates of *Escherichia coli* O157:H7 and subjected to a vacuum perfusion process to force (internalize) the bacterial cells into the intercellular spaces of the leaves. The leaves were treated with a 3-min water wash, a 3-min wash with a sodium hypochlorite sanitizing solution (300 ppm or 600 ppm), or various doses of ionizing radiation (0.25–1.5 kGy). Leaves were stomached to recover the internalized cells and survivors enumerated. The vacuum perfusion effectively forced bacteria into the leaf tissues. For spinach leaf pieces, neither water nor either of the sodium hypochlorite washes resulted in significant reductions of *E. coli* O157:H7 cells relative to the untreated control. For romaine lettuce leaf pieces, 300 ppm and 600 ppm each produced less than 1 log reduction, while water wash was not effective. Ionizing radiation, in contrast, significantly reduced the pathogen population in a dose-dependent manner, with reductions of 4 log units (romaine lettuce) or 3 log units (spinach) at the highest dose tested. The D10 value (the amount of irradiation necessary to reduce the population by 1 log) was higher for *E. coli* O157:H7 cells internalized in spinach leaves (0.45 kGy) than for cells internalized in romaine lettuce leaves (0.39 kGy). This study has shown that, unlike chemical sanitizers, ionizing radiation effectively eliminates internalized *E. coli* O157:H7 cells from leafy green vegetables, and that the pathogen is significantly less sensitive to radiation in spinach leaves than in romaine lettuce leaves.

T2-06 Concentration and Detection of *Salmonella* in Sprouted Seed Spent Irrigation Water Using Micro-filtration Coupled with Flow through ELISA

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Introduction: Sprouted seeds (alfalfa, bean sprouts) have been implicated in over 34 outbreaks of foodborne illness, principally involving *Salmonella*. Testing spent irrigation water for the presence of pathogens has been recommended as a key food safety intervention. However, laboratory based screening techniques

are time consuming and expensive. A more suitable alternative would be the on-site screening of composite water samples taken from sprouting seed beds.

Purpose: To develop an integrated pathogen concentration and detection system that enables rapid screening of large volumes (10 liters) of spent irrigation water.

Methods: *Salmonella* was concentrated from 10 liter volumes of water using tangential flow membrane filtration (0.2 μm pore size). The retinate was passed over a cellulose acetate membrane modified with anti-*Salmonella* antibody. A secondary antibody enzyme (alkaline phosphatase) was reacted with the captured *Salmonella*. Visualization of the secondary antibody binding was performed by addition of NBT/BCIP enzyme substrate.

Results: The tangential flow membrane filtration step was optimized in terms of flow rate and transmembrane pressure to enable maximum recovery of *Salmonella*. By using the optimized filtration step it was possible to achieve a 1000 fold concentration of *Salmonella* (2 log CFU/ml concentrated to 5 log CFU/ml). The retinate was passed (0.88 ml/min) over the antibody modified cellulose acetate membrane and reacted with the enzyme conjugate. Upon addition of enzyme substrate, a visual color change on the membrane could be detected within 10 min. In comparison, negligible color development was observed for non-inoculated controls. The total analysis time for filtration and detection was less than 3 h.

Significance: An integrated concentration and detection system has been developed for screening large volumes of spent irrigation water for the presence of pathogens. The system can be readily automated for on-site water testing by sprout producers.

T2-07 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 that oysters must be refrigerated within a defined timeframe, which changes seasonally. Even the more stringent summer guidelines allow for extended time in which oysters are held at ambient temperatures, providing an opportunity for bacterial proliferation. Despite recent risk assessment exercises, there is actually little information about how *V. parahaemolyticus* and *V. vulnificus* levels change during commercial harvesting.

Purpose: The purpose of this study was to characterize how extended deck storage of shellstock, which frequently occurs during commercial harvesting, affects the levels of *V. vulnificus* and *V. parahaemolyticus*.

Methods: Oysters were harvested four times at 3-month intervals from three separate Louisiana coastal locations. Samples were taken at the beginning of the harvest (time 0) and then at 2.5, 5.0, 7.5, and 10 h intervals after being held on the boat deck during harvest. Oyster samples were placed on ice immediately after collection and processed for microbiological analysis within 24 h of collection. *V. parahaemolyticus* and *V. vulnificus* levels were determined by colony lift DNA hybridization and representative isolates were collected and subjected to phenotypic and genotypic analysis. The relationships between *V. parahaemolyticus* and *V. vulnificus* levels, time on boat deck, and season were determined by analysis of variance (ANOVA).

Results: The levels of *V. vulnificus* in oysters increased by up to one log over the 10 h harvest period. Specifically, during the summer season, levels averaged about 10^2 CFU/g at time 0, and increased to 7×10^3, 2×10^4, and 3×10^4 CFU/g after oysters remained on the boat deck for 5, 7.5, and 10 h, respectively. During the winter, *V. vulnificus* was non-detectable (<10 CFU/g) in oysters until they had been stored on boat deck for 5 or more h, at which point the levels increased to 10^2 CFU/g. A significant increase in *V. vulnificus* levels occurred between 5.0 and 7.5 h in oysters stored on the boat decks during spring and fall season harvests. A similar trend was observed for the levels of *V. parahaemolyticus* in oysters during harvest.

Significance: Comparatively speaking, our data for *V. vulnificus* levels exceeds estimates used in recent risk assessments, while the data for *V. parahaemolyticus* confirms previously cited estimates. Taken together, this study provides information about how levels of *V. vulnificus* and *V. parahaemolyticus* change during commercial harvesting of Gulf Coast oysters.

T2-08 Incidence of *Listeria* spp. and *Listeria monocytogenes* in Blue Crab Meat (*Callinectus sapidus*) and Blue Crab Processing Plants

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Listeria species are widely distributed in many natural and man-made environments. Epidemiological studies performed in recent years have demonstrated that *L. monocytogenes* is an important pathogen transmitted by food. However, little information is available about the incidence of *Listeria* spp. and *L. monocytogenes* in blue crab meat and crab processing plants in Maryland even though crab meat is a Ready-to-Eat food and is economically the most important product in Maryland's seafood industry. The purpose of this study was to determine the incidence of *Listeria* spp. and *L. monocytogenes* in raw crab, finished product and environmental sponge samples. Samples were collected monthly from seven processing plants in Maryland during the crabbing season (May–November 2006). A total of 272 raw crab, 344 finished product and 344 environmental sponge samples were analyzed for *Listeria* spp. and *L. monocytogenes* by the US Food and Drug Administration bacteriological analytical method. Presumptive *L. monocytogenes* was confirmed by polymerase chain reaction using the BAX kit. Presumptive *Listeria* spp. were isolated from 19.1% of the raw crab, 19.2% of the finished product and 66% of the environmental samples. *L. monocytogenes* was isolated from 8.1% of the raw crab, 0.3% of the finished product and 5.8% of the environmental

samples. Among the environmental samples, *Listeria* spp. were found mostly in scrap barrels (86%), receiving docks (72.1%), raw crab coolers (69.8%), tables and gloves (60.5%) and cooked crab trays used for holding crabs prior to picking (65.1%). The most contaminated environmental sites for *L. monocytogenes* were raw crab coolers (20.9%) and receiving docks (13.9%). These results suggest that raw crabs are the primary source of *L. monocytogenes* contamination.

T2-09 DSC Use of Multiplexed Real-time PCR for the Detection of Pathogenic *Vibrio parahaemolyticus* in Oyster Homogenate

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Introduction: *Vibrio parahaemolyticus* (Vp) is a ubiquitous marine-dwelling bacterium that causes gastroenteritis and is of particular concern when raw shellfish are consumed. The presence of the thermostable direct hemolysin (tdh) gene and the thermostable direct hemolysin-related (trh) gene have been linked to pathogenic Vp strains. However, strains possessing these genes make up only a small fraction of the overall Vp population in the marine environment.

Purpose: The purpose of this study was to screen oyster homogenate enrichments for the presence of the tdh and trh genes and subsequently to isolate pathogenic Vp strains.

Methods: Oyster samples were harvested from three separate Louisiana Gulf Coast locations over a 12-month period during four sampling seasons spaced approximately 3 months apart. Cultural enrichments done on 10 g of oyster homogenate with overnight incubation at 37°C were tested for the presence of tdh, trh, and the thermolabile direct hemolysin (tlh) gene by use of a multiplexed real-time PCR assay with an internal amplification control. Enrichments positive for the tdh and/or trh genes were plated and a colony lift hybridization was performed to isolate pathogenic Vp strains.

Results: The gene targets associated with pathogenicity (tdh and/or trh) were detected by real-time PCR in 16.7, 58.9, 56.7 and 40.0% of the 10 g oyster homogenate enrichments during the winter, spring, summer and fall seasons, respectively. During seasons in which water temperatures and Vp levels were highest (spring and summer), no pathogenic isolates were obtained by colony lift hybridization. During the winter sampling season, three pathogenic isolates were obtained, while in the fall sampling season, one isolate was obtained.

Significance: Despite the apparent presence of pathogenic Vp strains during all seasons, their isolation is complicated. We hypothesize that these pathogenic strains may be poor competitors, particularly in the presence of high numbers of non-pathogenic Vp strains. Further work is in progress to test this hypothesis.

T2-10 The Effect of Salt, Smoke Compound and Storage Temperature on the Growth of *Listeria monocytogenes* in Simulated Smoked Salmon

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Smoked salmon can be contaminated with *Listeria monocytogenes*. It is important to develop effective control measures by identifying factors that would control *L. monocytogenes* growth in smoked salmon. The purpose of this study was to evaluate the effect of salt, a liquid smoke, storage temperature and their interactions on *L. monocytogenes* growth in simulated smoked salmon. A 6-strain mixture of *L. monocytogenes* (10^2–10^3 CFU/g) was inoculated into cooked salmon containing 0–10% NaCl and 0–0.4% liquid smoke (0–34 ppm phenol), and the samples were stored at temperatures ranging from 0 to 25°C. Lag phase duration (LPD, h), growth rate (GR, log CFU/h) and maximum population density (MPD, log CFU/g) of *L. monocytogenes* in salmon as affected by the concentrations of salt and phenol, storage temperature and their interactions were analyzed. Results showed that *L. monocytogenes* was able to grow in salmon containing the concentrations of salt and phenol commonly found in smoked salmon at the prevailing storage temperatures. The growth of *L. monocytogenes* was affected significantly ($P < 0.01$) by salt, phenol, storage temperature and their interactions. Higher levels of salt or lower storage temperatures extended the LPD and reduced the GR. Higher levels of phenol extended the LPD of *L. monocytogenes*, particularly at lower storage temperatures. However, its effect on reducing the GR of *L. monocytogenes* was observed only at higher salt concentrations (> 6%) at refrigerated and mild abuse temperatures (< 10°C). The MPD, which generally reached 7–8 log CFU/g in salmon that supported *L. monocytogenes* growth, was not affected by the salt, phenol and storage temperature. The data from this study would be useful for estimating the behavior of *L. monocytogenes* in smoked salmon.

T2-11 DSC Use of Antimicrobial Packaging Films and Edible Coatings to Control the Growth of *Listeria monocytogenes* on Cold-smoked Salmon

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Introduction: The relatively high incidence of *Listeria monocytogenes* (6–36%) in cold smoked salmon (CSS) is of concern as CSS is a Ready-to-Eat product. No post-processing measures are currently available to control this pathogen in CSS.

Purpose: The objectives of this study were to (a) screen binary combinations of nisin with individual GRAS preservatives incorporated in antimicrobial packaging films and edible coatings to control the growth of *L. monocytogenes* on CSS and (b) evaluate the efficacy of tertiary combinations incorporating nisin with the two most effective preservatives at low and high concentrations.

Methods: Slices of CSS were surface-inoculated with a five-strain cocktail of *L. monocytogenes* to a final density of 5-log CFU/cm^2. Samples were either packaged in antimicrobial packaging films (LDPE film coated with different antimicrobials) or coated with antimicrobial-containing edible solutions. Nisin alone (500 IU/cm^2) and the following different binary combinations of antimicrobials were incorporated into plastic films and edible coatings: nisin with sodium

diacetate (0.25%), sodium lactate (0.3%), sodium benzoate (0.1%), or potassium sorbate (0.3%). Combinations of nisin with the two most effective preservatives, sodium benzoate and potassium sorbate, were subsequently studied. Samples were stored at room temperature for 10 days and enumerated for *L. monocytogenes* every 2 days.

Results: After 10 days of storage, *L. monocytogenes* populations on all treated slices were ca. 0.2–3.1 and 0.7–3.6 log CFU/cm^2 lower than on control samples for antimicrobial packaging films and edible coatings, respectively. Combinations of nisin, potassium sorbate and sodium benzoate were the most effective against *L. monocytogenes* for antimicrobial packaging films and edible coatings. In addition, edible coatings had higher inhibitory effect than the corresponding packaging films, although the difference was not statistically significant ($P > 0.05$).

Significance: This research demonstrates the potential for using packaging films and edible coatings incorporating potassium sorbate and sodium benzoate to enhance the antilisterial effectiveness of nisin on CSS.

T2-12 DSC Spoilage and Shelf Life of Refrigerated Reduced Oxygen Packaged Atlantic Croaker (*Micropogonias undulatus*)

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Introduction: Recently, alternative ways of lengthening the shelf life of fresh fish products have been investigated; one of the most notable is the use of vacuum packaging. The FDA has mandated that refrigerated fishery products be packaged in film providing an oxygen transmission rate (OTR) of 10,000 cc/m^2/24 h in order to allow microbial spoilage before possible *Clostridium botulinum* toxin development. Additional research is needed, however, to determine if films with lower OTRs can increase the shelf life of fresh fish products without increasing safety risks from possible toxin formation from *Clostridium botulinum*.

Purpose: The purpose of this study was to determine the effects of storage temperature and film OTR on microbial spoilage and sensory spoilage for refrigerated raw vacuum packaged croaker fillets.

Methods: Raw croaker fillets were vacuum packaged in oxygen-permeable films (OTR of 10,000 cc/m^2/24 h or 3,000 cc/m^2/24 h) and stored at either 4°C or 10°C. For the microbial shelf-life study, psychrotrophic plate counts were determined for up to 12 days storage; spoilage was defined as 10^7 CFU/g. For the sensory shelf-life study, the odor difference was compared with a control sample, and spoilage was defined as a score of 3 or more.

Results: For both microbial and sensory spoilage, the interaction between temperature and rate of spoilage was statistically significant ($P < 0.05$). For example, shelf life of the fish was increased by 4 days when stored at 4°C versus 10°C. For both microbial and sensory spoilage the main effects of film OTR were not statistically significant ($P > 0.05$).

Significance: These data suggest that the rate of microbial and sensory spoilage of croaker fillets is significantly affected by storage temperature, but microbial and sensory spoilage may not be significantly affected by film OTR.

T3-01 Adhesion Forces of *Listeria monocytogenes* Scott A Biofilms Exposed to Surfactant Micellar-encapsulated Eugenol and Carvacrol Solutions as Measured by Atomic Force Microscopy

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen that can form biofilms that are more resistant to disinfectants than planktonic cells.

Purpose: The purpose of this study was to measure the adhesion forces of a silicon Atomic Force Microscopy (AFM) cantilever interacting with *L. monocytogenes* biofilms before and after treatment with micellar-encapsulated essential oils.

Methods: Biofilms of *L. monocytogenes* Scott A were grown on stainless steel coupons in a CDC biofilm reactor at 32°C for 48 h. Coupons containing biofilms were placed in treatment solutions (0.9% eugenol- or 0.7 % carvacrol-loaded Surfynol® surfactant micelles) or water for 2 min, followed by washing in phosphate buffered saline (PBS). After treatment, biofilms were air dried for 60 min before imaging with a Veeco CP-II AFM with a Multi 75Al cantilever in contact mode with a spring constant of 3 N/m and a scan rate of 1 Hz using 256 samples per line. Data was evaluated using one-way analysis of variance (ANOVA, SigmaStat 10.0).

Results: High-resolution images of biofilms were obtained using atomic force microscopy (AFM). Force-distance curves between the tip of the cantilever and the surface of the biofilm showed that the adhesion forces measured were significantly ($P < 0.05$) lower after antimicrobial treatment. The average adhesion forces observed after eugenol and carvacrol treatment was -23.00 and -24.54 nN, respectively, while the average measured adhesion force of non-treated biofilms was -64.48 nN. The distribution of adhesion forces measured varied greatly prior to treatment (0 to -160 nN); however, after treatment with both eugenol and carvacrol, all pull off events were under -60 nN.

Significance: Treatment with antimicrobial-loaded surfactant micelles leads to a modification of the physical surface of *L. monocytogenes* biofilms, possibly due to adsorption of surfactant, diffusion of antimicrobial into the biofilms or removal of extracellular polymeric substances surrounding cellular surfaces.

T3-02 Oregano Essential Oil: A Potential Food Industry Disinfectant

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Introduction: Surfaces and equipment used for food handling and processing are recognized as sources of microbial contamination and recontamination, particularly when many food pathogenic and spoilage bacteria are known to remain viable even after cleaning and disinfection. Moreover, biofilm formation and bacterial resistance to disinfectants have imposed a strong selective pressure for disinfectants within the food industry.

Purpose: The purpose of this study was to evaluate the ability of a natural antimicrobial agent (water soluble fractions of oregano essential oil) and a chemical (commercial) disinfectant to sanitize (a) the environment (cold rooms), (b) the equipment (tables, stuffing and mincing machines) and (c) the utensils (knives) of a meat processing plant.

Methods: The efficacy of the two tested disinfectants was assessed by swabbing a delimited surface area before cleaning, after cleaning and after disinfection, according to the routine cleaning/sanitation program of the food processing plant. Following these steps, microbiological testing was carried out on the swabbed surfaces. Differences in the number of bacteria remaining on the surfaces after each particular treatment were compared by ANOVA.

Results: Sanitation was effective in the following order: stuffing machine > mincing machine > cutting machine > knives. The microbial populations of yeasts/molds, and pseudomonads exhibited similar sensitivity to the two tested disinfectants. When treated with the natural agent, *Enterobacteriaceae* were reduced to below the detection limit on knives and cutting machine, in contrast to results with chemical treatment. Conversely, the use of chemical disinfectant on all tested equipment surfaces was slightly more effective against microbial populations of lactic acid bacteria and staphylococci, compared to the natural antimicrobial agent.

Significance: Our results suggest that natural antimicrobial agents may be equally effective for sanitation purposes as the currently used chemical ones.

T3-03 Fate of *Escherichia coli* O157:H7 When Exposed to Sub-lethal and Lethal Concentrations of Common Industrial Sanitizers

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Introduction: *Escherichia coli* O157:H7, a familiar foe of the food industry, is capable of surviving hostile and harsh environments, which can result in contamination of food or in foodborne illness or outbreak. As the majority of the responsibility to provide a safe product falls on the food industry, it is essential to maintain an effective sanitation program.

Purpose: The purpose of this study was to evaluate the ability of *E. coli* O157:H7 to survive and adapt to the chemical stress of sanitizers common in the food industry.

Methods: Planktonic and adherent isolates from *E. coli* O157:H7 ATCC 700599 and *Escherichia coli* O157:H7 FSIS 063-93 were exposed to a chlorine, an iodophor, a quaternary ammonium and a peroxyacetic acid compound at room temperature for five min for three consecutive days. Cells were recovered on violet red bile agar and their minimum inhibitory concentration (MIC) was reassessed. Mean log kill for each strain and sanitizer for planktonic and adherent bacterial cells was analyzed using SAS.

Results: There were statistically significant differences ($P < 0.05$) in the ability of the sanitizers to effectively reduce bacterial counts for both planktonic (> 5 log) and adherent (> 3 log) cells for both *E. coli* strains. The iodophor compound was the only sanitizer found to be effective on both planktonic and adherent bacterial isolates. Additionally, methodology was found to be important, as lack of recovery by plate count methodology did not correlate with death of the organism (growth in Lauryl Sulfate broth measured at 580 nm).

Significance: These data stress the importance of proper cleaning and sanitation procedures in the food industry and suggest that methodology for recovery of bacteria is important.

T3-04 Chlorine Sensitivity of Feline Calicivirus, a Surrogate of Norovirus

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Introduction: Though chlorine disinfectants are thought to be effective for norovirus, recommended concentration is usually higher than 100 ppm. On the other hand, several outbreaks caused by drinking and recreational water were associated with failure of chlorination, the regulations and the standards of which are not higher than 5 ppm. Because of lack of the infection systems for norovirus, most of the studies that indicate chlorine sensitivity were done by using surrogates, such as feline calicivirus (FCV).

Purpose: The chlorine sensitivity of FCV was reevaluated by changing chlorine demand of the mixture in which FCV was treated with the disinfectant.

Methods: FCV was propagated in Crandell feline kidney cells and collected by differential centrifugation (crude FCV) followed by partial purification by centrifugation on 25% sucrose cushion. These fractions were treated with various concentrations of sodium hypochlorite.

Results: Apparent sensitivity of the crude FCV depended on the volume of reaction mixture and the reduction of host cell debris. Infectivity of the partially purified FCV was reduced by more than 4.6 log by 5 min treatment with 0.3 ppm free chlorine.

Significance: The results indicated a higher sensitivity of FCV to free chlorine than has been previously reported. If chlorine sensitivity of norovirus is similar to that of FCV, 1 ppm of free chlorine should be sufficient to control norovirus. This suggests that the regulations and the standards of chlorine concentration may be effective in circumstances with low chlorine demand, such as drinking and recreational water.

T3-05 Sensitivity of *Escherichia albertii* to Food Preservation Treatments

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Introduction: *Escherichia albertii* is a potential foodborne pathogen because of its ability to cause diarrheal disease in humans. *E. albertii* may be mischaracterized as *E. coli* O157:H7 because of their similar appearance on selective media.

Purpose: The sensitivity of *E. albertii* to food preservation treatments — heat, acid and pressure — was determined.

Methods: Five strains of *E. albertii* (9194, 10457, 10790, 12502, and 19982), wild-type *E. coli* O157:H7, *rpoS*-deficient *E. coli* O157:H7, *Shigella boydii*, and *S. flexneri* were individually exposed to heat (56°C), acid (pH 3.0, 2 h at 37°C), and hydrostatic pressure (500 MPa, 5 min at 22°C). Populations of strains were recovered on TSA and significance ($P < 0.05$) of differences in $D^{56°C}$ values and in populations exposed to acid and pressure were calculated.

Results: $D^{56°C}$ values of *S. boydii* and wild-type *E. coli* O157:H7 were significantly greater than those of all other strains. $D^{56°C}$ values of *E. albertii* strains 10457 and 19982 were significantly greater than those of strains 9194, 10790, and 12502. Wild-type *E. coli* O157:H7 and *Shigella* spp. had greater acid tolerances than all *E. albertii* strains. Strains 9194, 10790, and 12502 displayed greater acid tolerance than strains 10457 and 12502. After 5 min of pressure treatment, cells of wild-type *E. coli* O157:H7 and *S. flexneri* were significantly more tolerant than those of *E. albertii* strains. Strain 19982 had a significantly higher pressure tolerance than strain 12052. For all treatments, no strain of *E. albertii* was more tolerant than wild-type *E. coli* O157:H7, but all strains showed significantly greater acid tolerance than *rpoS*-deficient *E. coli* O157:H7.

Significance: This study is the first to report the sensitivities of *E. albertii* to food preservation treatments. It suggests that there is diversity in the sensitivities of *E. albertii* strains to these treatments, but none were less sensitive than wild-type *E. coli* O157:H7.

T3-06 DSC On the Role of Colicin E1 against Gram-positive Bacteria

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Colicins are a class of gram-negative bacteriocins produced by, and effective against, *Escherichia coli* and closely related species. Colicin E1 (ColE1) is composed of three functional domains, which collectively cause a pore-forming effect on targeted bacteria. The binding and translocation of ColE1 into targeted gram-negative bacteria is highly receptor-specific and coordinated; however, the pore-forming activity on the bacterial membrane is less specific. The primary purpose of this study was to investigate the effectiveness of ColE1 against *Listeria monocytogenes*, a gram-positive, foodborne pathogen. Individual strains of *L. monocytogenes* were examined in TSB containing 0, 0.1, 1, or 10 μg ColE1. While strain differences in sensitivity to ColE1 existed, growth was significantly reduced in all strains at the 0.1 μg/mL dose. In an effort to deduce the mechanism of ColE1 against *L. monocytogenes*, the most susceptible *L. monocytogenes* strains from the in-vitro study were subjected to flow cytometric analysis in comparison to *E. coli* K-12, an extremely ColE1-susceptible strain. Bacteria were grown to ~ 4 log CFU/mL and incubated with either 0.1 or 1 μg/mL ColE1. Scanning measurements were conducted at 0, 30 and 60 min post-ColE1 treatment by use of DiBAC4(3), a membrane potential-sensitive probe. ColE1 caused similar membrane-depolarizing effects in *E. coli* and *L. monocytogenes*, implying analogous pore formation in the two bacteria. Binding and movement of ColE1 within cells were visualized using immunegold labeling and transmission electron microscopy. To elucidate possible ColE1 receptors within *L. monocytogenes* aiding in ColE1 activity, bacterial lysates were separated using 12% SDS-PAGE and transferred to PVDF-membranes. Biotinylated ColE1 was over laid on membranes after blocking, and blots were detected using streptavidin-hp. Two distinct proteins (M.W. = 65, 40kD) were detected in the *L. monocytogenes* lysate, uncovering proteins within *L. monocytogenes* that ColE1 has high affinity for. Characterization of these proteins will aid in understanding the mechanism of ColE1 in gram-positive bacteria.

T3-07 DSC Incidence of Contaminating Microbial Species throughout Manufacture and Ripening of São Jorge – A Portuguese Traditional Cheese from Raw Milk

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Introduction: Prerequisites for good, safe and appealing dairy products include cow milk of high microbiological and chemical quality. São Jorge, an Appélation d'Origine Controlée cheese, is manufactured from raw milk, in small dairy farms scattered thoughout the islands of Azores (Portugal). This cheese accounts for 51% of the whole production of traditional cheeses in Portugal, and has already found an important market in the USA and Canada.

Purpose: In order to ascertain the sanitary conditions prevailing during manufacturing, levels of contaminating bacteria were assessed in 70 raw milk samples and in 70 cheese samples, as well as on working surfaces of 7 certified dairy plants. An inspection checklist for food safety and sanitation (consisting of 15 checkpoints, related to sanitation control of facilities and equipment, as well as personal hygiene) was also used in the assessment of all dairy plants.

Methods: Microbiological analyses included the determination of total viable counts (on PCA medium), as well as viable counts of *Salmonella* (on *Salmonella* chromogenic agar medium), *Enterobacteriaceae* (on VRBGA medium) and *Micrococcaceae* (on MSA medium). API20 and APIStaph were subsequently used for identification of *Enterobacteriaceae* and *Micrococcaceae* organisms.

Results: Total and *Enterobacteriaceae* viable counts ranged from 6–8 log CFU/ml and 5–6 log CFU/ml, respectively, in raw milk, whereas they ranged from 7–8 log CFU/g and 0–2 log CFU/g, respectively, in 4-month old cheeses. No *Salmonella* spp. was detected in any samples. *Micrococcaceae* viable counts ranged from 5–6 log CFU/ml and 3–4 log CFU/g, in raw milk and 4-month old cheeses, respectively. *Enterobacteriaceae* organisms were identified as *Klebsiella oxytoca* (24% of isolates), *Klebsiella cloacae*, *Klebsiella pneumoniae*, *Enterobacter sakazakii* (18% each), and *Escherichia coli* (6%). Two species, *Klebsiella ornithinolytica* and *Klebsiella terrigena*, were detected only in raw milk. Among *Micrococcaceae*, *Staphylococcus sciuri* was the most common (29% of isolates), followed by *Staphylococcus epidermidis* (26%) and *Staphylococcus aureus* (5%), whereas *Staphylococcus saprophyticus* (2%) was found only in 4-month-old cheeses.

Significance: The high counts of *Enterobacteriaceae* in cheese and the presence of *S. aureus* in raw milk reveals poor milk-handling conditions; the latter organism was also detected on surfaces of 5 out of 7 dairy plants. The results from the checklist support the need for improving sanitation in dairy facilities and equipment. In general, inappropriate or insufficient hand-washing facilities were observed, and good water supply and educated workers were absent. HACCP programs are thus urged for essentially all dairy farms in that region.

T3-08 Cleaning Validation for Allergen Removal: Food Factory Case Studies

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Introduction: Food manufacturers have a duty of care to either minimize the likelihood of cross contamination with allergens or to warn consumers if products may contain allergens. Validation of cleaning regimes is required where HACCP plans identify cleaning as a critical control to ensure adequate removal of allergens. To examine the efficacy of cleaning regimes, testing can be performed on final products and environmental swabs using allergen detection tests, such as enzyme-linked immunosorbent assays (ELISAs).

Purpose: The aim of this work was to demonstrate how validation of cleaning regimes can be conducted by use of allergen detection tests in food factories.

Methods: Case studies were conducted in food factories where clean-in-place (CIP) and an open conveyor belt system were cleaned to remove allergens. Samples, in the form of environmental swabs (e.g., from conveyor belts, balances, trays, filler nozzles and balance tanks), rinse waters and products were taken following manufacture of a product known to contain an allergen; after the normal cleaning regime for the factory; and during a subsequent run of non-allergen containing product. The samples were tested by use of commercially available test kits for detection of allergenic ingredients (Neogen, Tepnel, and ELISA Systems), hygiene tests (ATP and protein, Biotrace) and a general protein assay (Pierce) where appropriate.

Results: Tests to ensure that the allergen detection kits were able to detect the allergen where it was known to be present were positive. Cleaning was shown to decrease environmental levels of allergen, although traces of allergen remained in some instances. No cross contamination was detected in non-allergen products manufactured following a run of allergen containing product, after a clean had taken place.

Significance: Validation of cleaning regimes for allergen removal has been demonstrated in CIP and open conveyor-belt systems. Comparisons were made between allergen detection kits and routine factory hygiene assessments.

T3-09 Effect of Cleaning Fluids on Detection of Allergens

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Introduction: Validation of cleaning regimes and laundry processes are two ways of demonstrating that adequate controls are in place to manage allergens in food processing environments. Allergen detection tests, such as enzyme-linked immunosorbent assays (ELISAs), can be used as part of this; however, the effect of cleaning fluids on the performance of these tests is unknown.

Purpose: The aim of this work was to determine whether cleaning fluids commonly used in food manufacturing environments affect the ability of ELISA tests to detect allergens. Work was also carried out to test whether allergen residues remain on clothing following laundering, and if garments contaminated with allergens could contaminate garments from allergen-free areas in the laundry.

Methods: Commercially available test kits for detection of allergenic ingredients (Neogen, R-Biopharm, Tepnel, and ELISA Systems) and a general protein assay (Pierce) were tested with cleaning fluids at different strengths (i.e., working concentration and 1/100 (v/v) dilution of the working concentration), and in factory case studies. Overalls at different stages of laundering were obtained from food manufacturers and tested for allergen residues.

Results: False positive and negative results were detected when specific test kits were used with particular cleaning fluids at different levels and in factory trials. However, no one test was influenced by all the cleaning fluids, nor did one cleaning fluid influence results of all the tests. Following laundering, allergen residues were removed from overalls, and no cross contamination with "dirty" overalls was observed in the factory case studies conducted.

Significance: The potential effect of cleaning fluids on the results of ELISA tests for allergens should be taken into account when testing rinse waters and swab solutions from the food factory environment. Testing of protective clothing for allergen residues following laundering should be considered as part of

the validation of allergen control measures in food factories.

T3-10 Sandwich ELISA for the Detection of Bovine Blood in Animal Feedstuffs

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Introduction: The feeding of ruminant proteins to ruminants is prohibited in most countries because the practice is thought to be responsible for the spread of fatal bovine prion diseases. However, currently available methods to detect ruminant blood products in heat-processed food and rendered feedstuffs are inadequate because they lack species specificity, and tissue specificity and because they are not based on a thermo-stable analyte.

Purpose: The objective of this study was to develop a sensitive and reliable sandwich enzyme-linked immunosorbent assay (ELISA) to detect bovine blood in rendered animal byproducts.

Methods: Adulterated samples were prepared by mixing bovine blood with porcine blood, and mixing spray-dried bovine plasma or whole bovine blood powder separately with spray-dried porcine plasma at 0%–10% adulteration levels. The liquid blood mixtures were subjected to sterilization (121°C for 15 min) or heating in a boiling water bath (15 min). Soluble proteins were extracted in 10 mM PBS from above samples for ELISA analysis. The sandwich ELISA employs monoclonal antibody (MAb) 6G12 as the capture antibody and biotinylated MAb 3D6 as the detecting antibody. Both MAbs recognize a 60 kDa thermo-stable protein in bovine blood.

Results: The sandwich ELISA is bovine specific and blood specific. The detection limit for the assay was 0.05% (v/v) for both autoclaved and cooked bovine blood in porcine blood samples. The detection limit was 0.1% (w/w) for spray-dried bovine plasma in spray-dried porcine plasma, and 0.5% (w/w) for whole bovine blood powder in spray-dried porcine plasma. The assay has an overall accuracy of 100%, and an overall variability of 1.28%.

Significance: This study reports the first MAb-based assay for the sensitive and reliable detection of bovine blood in both heat-processed feedstuffs and unprocessed raw materials, providing a useful regulatory tool for monitoring fraudulent labeling or contamination with bovine blood in a wide range of products.

T3-11 Pesticides Determination in Three Fruit-based Baby Foods Using Different Extraction Methods and Gas Chromatography

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Introduction: Pesticide residues represent one of the most important chemical contaminants in fruits and their processed products, including baby foods. Maximum residue limits (MRLs) in baby foods, which are considered complex matrices, are extremely low (≤ 0.01 mg/kg). Thus, there is intensive demand for further and reliable monitoring of pesticides at low levels, in products with such high (chemical) safety standards.

Purpose: To comparatively evaluate the matrix effects on the recovery of organophosphorus pesticides from non fatty baby foods as obtained with three rapid and relatively inexpensive extraction methods.

Methods: Fruit-based ready to eat baby food matrices (fruit juice: matrix1, fruits purée: matrix2, fruit cocktail+rice flour/starch+sugar: matrix3) were fortified with indicative levels (0.3, 0.1 and 0.01 mg/kg) of dimethoate, methamidophos, chlorpyrifos, methidathion and phosalone. Samples were prepared in triplicate by three extraction methods (ethyl acetate, acetone partition and dichloromethane) and analyzed by gas chromatography with nitrogen-phosphorus detector (GC-NPD). Recoveries were estimated by a single point determination (in comparison with solvent standards) and results were analyzed by 3-way ANOVA.

Results: Pesticides recoveries were affected ($P < 0.05$) by both the matrices and the extraction method. Approximately, the mean recoveries of all analytes were in the following decreasing order of matrices (ranges refer to ethyl acetate, dichloromethane and acetone partition method, respectively): matrix2 (26.4–117.9%, 50.1–183.0%, 33.5 206.0%) >matrix1 (44.3–76.3%, 42.2–99.6%, 51.4–142.9%)> >matrix3 (28.2–66.0%, 29.0–165.3%, 23.1–133.0%). Likewise, the examined methods may be ranked based on the extraction ability and analytes recoveries as follows: acetone partition>dichloromethane >>ethyl acetate. Ethyl acetate extracts showed large amounts of lipophilic compounds and integratable peaks of the polar methamidophos.

Significance: The data suggest that the more complex the formulation of baby food, the lower the pesticides recoveries. Therefore, the monitoring of pesticides in baby foods should be very careful, especially since underestimation of actual pesticides content seems inevitable under conventional methods and practices.

T3-12 Quantification of Mycotoxins Deoxynivalenol, Masked Deoxynivalenol and *Fusarium graminearum* Pigment in Wheat and Rice Samples Using a New LC-UV/MS Method

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Introduction: Tolerable limits set for deoxynivalenol (DON) do not consider DON conjugates such as deoxynivalenol-3-glucoside (D3G), which exists in cereals. These conjugates are metabolized to DON. Such masked mycotoxins and *Fusarium graminearum* pigments, such as the toxic aurofusarin reported in cereals, are not routinely analyzed.

Purpose: To quantify DON, D3G and the red Fusarium pigment in grain or grain cultures using a new liquid chromatography (LC) – mass spectrometry (MS) method. We also assessed extraction protocols to optimize analysis.

Methods: A two-way factorial on a randomized complete block was used to compare protocols: centrifugation and shaking using methanol: methylene chloride or acetonitrile: water (84:16 v/v). Wheat and rice samples were extracted with solvent filtered over C18 packing material and analyzed using LC-UV/MS. A C18 column using an isocratic mobile phase of 70% methanol was used to separate analytes. A UV detector and ESI in scan mode were the detection methods. Method validation included limit of detection (LOD) and limit of quantification (LOQ).

Results: The average DON was 3.4 mg/kg (± 4) in field wheat and 15.2 mg/kg (± 41) in rice culture. Deoxynivalenol-3-glucoside averaged 3.8 mg/kg (± 8.26) in field wheat samples and 5.4 mg/kg (± 8.6) in cultured rice. Neither DON nor D3G were observed in wheat cultures. Pigments averaged 3,109.6 mg/kg (± 3,714.0) in field wheat but were higher in wheat cultures (42,208.0 mg/kg ± 256,260.0) and rice cultures (7,115.8 mg/kg ±12,575.0). Recoveries were 96% (DON) and 70% (D3G), while LODs were 1 ng/ml (MS), 10 ng/ml (UV) and LOQs were 0.001 mg/kg (UV), < 0.001mg/kg (MS). Centrifugation with methanol: methylene chloride yielded more myco-toxins than shaking with acetonitrile: water.

Significance: This LC-UV/MS method demonstrates the presence of masked mycotoxins in foods. Common analytical tools can be used to quantify masked and parent mycotoxins as well as toxic pigments for risk assessment.

T4-01 DSC A Comparative Analysis of the Effect of Pasteurization and High-pressure Processing on the Stability and Infectivity of Bovine Rotavirus

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Introduction: Rotavirus is a major cause of severe gastroenteritis in human infants and animals, including diarrhoeal disease in calves. Rotaviruses are triple-layered viruses, with a genome of 11 segments of double-stranded (ds) RNA. The two outer capsid proteins, VP4 and VP7, must be functional for infectivity. Bovine rotavirus (BRV) has been detected in dairy cattle herds; thus it provides a potential source of contamination of dairy products. Development and validation of methods to assess the efficacy of processing techniques to control viral contamination of food products is needed.

Purpose: Pasteurization, the current gold standard in dairy processing, and high-pressure processing (HPP), a novel non-thermal food processing technique, were assessed with respect to their efficacy in inactivatating BRV.

Methods: A number of assays, including fluorescent focus neutralization assays, enzyme immunoassays (EIA) and real-time RT-PCR, were used to characterize the effect of pasteurization and HPP on BRV stability and infectivity.

Results: Heat treatment experiments replicating pasteurization showed a 2-log reduction in viral titres of BRV at 62°C for 15 s and a greater than 3-log reduction at 72°C for 15 s. Evaluation of HPP treatment on BRV stability was conducted using four different pressures (200, 300, 400 and 500 MPa) and four to seven time points. HPP of BRV resulted in a 3-log or greater reduction in viral titre at pressures 300, 400 and 500 MPa. Real-time RT-PCR results indicated that the mechanism by which HPP inactivated BRV particles was by removal of the outer capsid, converting triple-layered particles into non-infectious double-layered particles. However, at an increased pressure and time exposure (500 MPa for 480 s), HPP denatured the entire viral particle, releasing freed RNA into the supernatant.

Significance: HPP appears to be as effective as high temperature short time (HTST) pasteurization in its ability to reduce the infectious titre of BRV.

T4-02 DSC High Temperature Short Time (HTST) Processing Temperatures Have Surprising Effects on Fluid Milk Aerobic Plate Counts

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Introduction: The Grade "A" Pasteurized Milk Ordinance (PMO) mandates that all HTST pasteurized milk products be heated to a minimum of 72°C and held for at least 15 s. However, it is common practice for milk processing plants to exceed the time and temperature requirements set forth by the PMO. Recently, fluid milk processors have reported an unexpected increase in consumer complaints regarding milk spoilage after pasteurization temperatures have been increased above the minimum for HTST fluid milk products.

Purpose: To test the hypothesis that HTST pasteurization temperatures affect aerobic plate counts of fluid milk products over a 21 day shelf life.

Methods: 2% milkfat raw milk was heat treated at four different temperatures (72°C, 77°C, 80.5°C or 85°C) for 25 s. Milk samples (2 l) were collected aseptically from each pasteurization treatment and held at 6°C. Aerobic plate counts were performed on the pasteurized samples at 1, 7, 14 and 21 days post heat-treatment, using the spread plate method on brain heart infusion agar, followed by a 24-hour incubation at 32°C. Average values for aerobic plate counts calculated for each treatment and time-point were log transformed and compared using t-tests.

Results: Aerobic plate counts were significantly greater ($P < 0.05$) in milk pasteurized at 85°C than milk pasteurized at 72°C at 24 h post-processing. On average, milk pasteurized at 85°C exhibited a 0.91 log higher aerobic plate count versus the milk pasteurized at 72°C, at days 1, 7, 14 and 21 post-processing ($P > 0.056$).

Significance: Our data provide scientific evidence in support of the observation that milk processed at higher HTST temperatures has a shorter shelf life (i.e., higher bacterial numbers post-processing) than milk processed at a lower temperature. These results suggest the need to optimize HTST fluid milk processing conditions to maximize product shelf lives.

T4-03 Use of 3M™ Petrifilm™ Aerobic Count Plates for the Detection of *Geobacillus stearothermophilus* in UHT Milk

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Introduction: *Geobacillus stearothermophilus* is a spore-forming thermophilic bacterium that can survive defective ultra-high temperature processes. The spore of this microorganism is one of the most heat-resistant of aerobic microorganism spores. *G. stearothermophilus* is frequently isolated from milk and milk products. Thus, for quality control, the milk industry analyzes the presence of microorganisms such as this bacterium in UHT processed products. For this, time-consuming traditional methods are used, and new alternatives using more rapid methods are required.

Purpose: We examined the 3M™ Petrifilm™ AC Plates to determine their effectiveness in detecting this thermophilic microorganism in UHT milk.

Methods: *G. stearothermophilus* ATCC 9953 was stored at -81°C. UHT- processed milk samples were analyzed for sterility by determining the presence of mesophilic and thermophilic bacteria, including spore counts. Spores of *G. stearothermophilus* were produced in trypticase soy broth (TSB), with $MnSO_4$, $MgSO_4$ and $CaCl_2$ that was incubated at 42°C for 48 h. Spores were collected, washed and inoculated in different concentrations into commercially sterile UHT-milk. Treatments were incubated at 42°C, and samples were collected at 24, 48 and 72 h for bacterial detection. *G. stearothermophilus* was detected and enumerated by the 3M™ Petrifilm™ AC Plates and the traditional one-streak in agar method. The limit of detection was determined for both methods.

Results: When 101 spores were inoculated in the UHT–milk and incubated 24 h, the method using 3M™ Petrifilm™ AC Plates was able to detect growth at 6 h, whereas the one-streak in agar method detected no growth until 12 h. Similar results were observed when the inoculated UHT-milk was incubated for 48 and 72 h. ANOVA ($P \leq 0.05$) showed significant difference between treatments.

Significance: The 3M method using Petrifilm AC Plates is a more rapid and effective alternative for detection of *G. stearothermophilus* in UHT processed milk, compared to the traditional one-streak method.

T4-04 Efficiency of Milk Culture, Direct PCR, and Nested PCR and Comparison with Fecal Culture Based on Samples from Dairy Herds Containing Cows with Johne's Disease

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Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease (JD) in cattle and other farm ruminants, and also a suspected pathogen in Crohn's disease in humans. Development of diagnostic methods for MAP infection has been a challenge over past decades.

Purpose: Investigate the relationship between different methods of detection of MAP in milk and fecal samples.

Methods: A total of 212 milk samples and 157 feces samples were collected from 146 individual cows in 12 herds in Southwest Ontario. Culture, IS900 PCR and nested PCR methods were employed for detection of MAP in milk, and the results were compared with results of the fecal culture.

Results: Significant correlation was observed among the culture, direct and nested PCR as applied to milk samples ($P < 0.0001$). Fecal culture was significantly correlated with milk culture and milk nested PCR ($0.01 < P < 0.05$), but not with milk direct PCR ($P = 0.6672$). Chi-square tests confirmed this correlation. The animals with high numbers of CFU in fecal culture may not be detected by milk culture, and vice versa. The low level shedders had a much higher frequency than the higher level shedders. One-third of the positive animals would have been missed if only one culture method, instead of cultures of both milk and feces, had been used for diagnosis.

Significance: The shedding of MAP in feces and milk is not well synchronized. The low level shedders may be detected only by chance. Subsequently, the low level shedders may even be underestimated. Therefore, both milk and feces should be collected, or/and the sample should be duplicated, for the suspected individuals. The high degree of microbial inactivation that occurs during decontamination steps in culture methods may be compensated by using nested PCR, which showed the highest sensitivity among the methods we used.

T4-05 Effect of Milk Concentration and pH on Pressure Inactivation of *Listeria monocytogenes*

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Introduction: The composition of the suspending medium is known to affect the resistance of microorganisms to pressure. The composition of dairy products (e.g., solids concentration and pH) may have an effect on the survival of pathogens, such as *Listeria monocytogenes*, during pressure treatment.

Purpose: To assess the effect of pressure (500 MPa/10 min/20°C), skim milk powder (SMP), concentration (6, 9, 12 or 15%) and pH (4, 5, 6 or 7) on the survival and subsequent growth at 4°C of *L. monocytogenes*.

Methods: *L. monocytogenes* NCTC 11994 was inoculated (at approximately 10^7/ml) into solutions of varying concentrations of SMP (6–15%) and pH values (4–7). The samples were pressure-treated (500 MPa for 10 min at 20°C initial temperature) or left untreated (controls). All samples were stored at 4°C for up to 8 days. At intervals, surviving *Listeria* were

enumerated on trypticase soy agar containing 3% yeast extract. The experiment was repeated on 3 separate occasions.

Results: SMP concentration and pH did not significantly affect ($P > 0.05$) survival or growth of *L. monocytogenes* in the control samples. However, there were significant interactions between pressure, pH and SMP concentration in the pressure-treated samples. At pH 4 and 5, no surviving organisms were detected thoughout storage in any sample (> log 6 reduction). At pH 6 and 7, some *L. monocytogenes* survived the pressure treatment and numbers increased significantly ($P < 0.01$) during storage, especially at the higher SMP concentrations.

Significance: Medium pH can have a significant effect on the survival of *L. monocytogenes* during pressure treatment of skim milk. This fact needs to be taken into consideration when devising pressure-treatment regimes to improve the microbiological safety of dairy products.

T4-06 DSC Prevalence of Target Pathogens in Farmstead Raw Milk Destined for Cheesemaking

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US federal regulations do not regulate the presence of pathogens in raw milk used for the manufacture of raw milk products. This study evaluated the overall milk quality and prevalence of four target pathogens in raw milk destined for cheesemaking. Raw milk samples were collected weekly (June–September) from 11 farmstead cheese operations manufacturing raw milk cheese from bovine (5), caprine (4), and ovine (2) milks. Overall quality was determined though standard plate (SPC) and total coliform counts (CC), as well as somatic cell counts (SCC). Additionally, samples were screened for *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* sp., and *Escherichia coli* O157:H7. For quantitative detection, raw milk was directly plated on chromogenic agar media. Qualitative detection was conducted using the automated PCR (DuPont™ Qualicon BAX® Q7). Overall, 96.8% of samples had SPC < 100,000 CFU/ml, 42.7% of which were <1000 CFU/ml. Although no federal standards exist for coliform levels in raw milk, 61% of samples tested were within pasteurized milk standards under the Pasteurized Milk Ordinance (PMO) at <10 CFU/ml. All bovine milk samples were within the limits of the PMO for SCC, and 88% met the stricter European Union regulations. Furthermore, 98.5% of all small ruminant samples complied with PMO standards for caprine milk. Of the 11 farms, 8 (73%) were positive for *S. aureus*, detected in 35% (46/133) of samples at an average level of 25 CFU/ml. *L. monocytogenes* was isolated from 2.26% (3/133) of samples (all bovine), two of which were from the same farm. *E. coli* O157:H7 was recovered from 1 sample (0.75%) of caprine milk. *Salmonella* sp. were not recovered from any samples. These data indicate that milk intended for artisan cheesemaking was of high microbiological quality, and form part of an exposure assessment and risk reduction plan to promote continuous production of microbiologically safe artisan cheese.

T4-07 DSC Elimination of *Listeria monocytogenes* in a Soft Cheese, Fromage Blanc, Using Natural Processing Methods and Formulation Changes

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Introduction: *Listeria monocytogenes* may cause severe and sometimes deadly illness in certain at-risk individuals. This pathogen survives on processing surfaces despite sanitation measures and may contaminate milk used for soft cheese making. There is need for new hurdle technologies to eliminate *L. monocytogenes* in soft cheeses, especially methodologies that allow the product to remain naturally produced.

Purpose: The purpose of this study was to evaluate the ability of different natural processing and formulation treatments, alone and in combination, to eliminate *L. monocytogenes* in soft-cheese making.

Methods: A cocktail of *L. monocytogenes* strains (J1-110, R2-502, C1-056) was used to contaminate (10^6 CFU/ml) pasteurized milk used in making soft cheese produced using standard methods. Different treatments were implemented, including addition of extra starter culture (1.25 g rather than 1.00 g), high temperature draining (45°C instead of 22°C), addition of differing levels of additive grade nisin (125 ppm, 250 ppm, 500 ppm), and combinations of these treatments into hurdle methods. *Listeria monocytogenes* was recovered on MOX immediately after packaging cheese. The soft cheese samples were also analyzed by measuring pH, fat content and protein content, using standard methods; these data and the resulting plate counts were compared by use of ANOVA.

Results: No statistically significant differences ($P > 0.05$) were found between the reported counts of *L. monocytogenes*. The treatments could not eliminate the organism, but reduction was seen, with the greatest reduction (1 log) seen in the combined treatments of additional starter culture and high temperature draining. Statistically significant differences ($P < 0.05$) were seen between pH, fat content, and protein content; however, the differences in sensory characteristics were not distinguishable.

Significance: The data suggests that the use of these treatments in Fromage Blanc, when used in combination with other methods, can offer reduction but not always elimination of *L. monocytogenes*. Additional hurdle treatments may be necessary to result in full elimination of *L. monocytogenes*.

T4-08 Prevalence and Molecular Ecology of *Listeria monocytogenes* in Retail Food Establishments

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that annually causes approximately 500 deaths in the United States. Estimates indicate that 1–10% of Ready-to-Eat foods may contain *L. monocytogenes*; however, the prevalence of *L. monocytogenes* in retail environments and the transmission of the pathogen to foods in these environments is not well understood.

Purpose: The goal of this study was to determine the prevalence of *L. monocytogenes* and specific *L. monocytogenes* molecular subtypes among foods and various environmental locations within retail establishments.

Methods: Food and environmental sponge samples were collected from retail establishments and selectively enriched. Samples were screened for *L. monocytogenes* using the VIDAS LMO II (bioMérieux) method and presumptive positive samples were plated onto selective agar. Typical colonies were confirmed by use of standard methods and subtyped by automated ribotyping (Qualicon).

Results: From 121 establishments, there were 185 food and 1158 environmental samples, from which 5 (2.7%) and 151 (13%) *L. monocytogenes* isolates were, respectively, obtained. There were a total of 73 establishments with at least one environmental isolate of *L. monocytogenes* and 46 with more than one isolate. All location types had at least one sample containing *L. monocytogenes*; however, 58.9% of the environmental isolates were obtained from various floor drains. The five food *L. monocytogenes* isolates were represented by three ribotypes, while the 151 environmental isolates were represented by 30 ribotypes. In 2 establishments, the food isolate ribotype was indistinguishable from at least one environmental *L. monocytogenes* isolate. There were 27 establishments with multiple environmental isolations of the same ribotype from different sample locations.

Significance: While *L. monocytogenes* was readily isolated from some retail environments, the organism was less frequently isolated from Ready-to-Eat food products. Molecular subtype data indicate the potential for specific *L. monocytogenes* subtypes found in retail environments to contaminate foods handled or processed within those establishments.

T4-09 Penetration of *Salmonella* Enteritidis and *S.* Heidelberg Strains into Egg Yolks during 36-hour Ambient Temperature Storage

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Although *Salmonella* deposition inside yolks is uncommon in naturally contaminated eggs, migration though the vitelline membrane into the nutrient-rich yolk contents could enable rapid bacterial multiplication. Egg refrigeration restricts both penetration and growth, but a recently proposed national S. Enteritidis control program would allow unrefrigerated ambient temperature storage of eggs on farms for up to 36 h. The present study used an in vitro egg contamination model to assess the ability of small numbers of four S. Enteritidis strains and four S. Heidelberg strains to penetrate the vitelline membrane and multiply inside yolks during 36 h of storage at either 20° or 30°C. After inoculation onto the exterior surface of the vitelline membrane, all eight *Salmonella* strains penetrated to the yolk contents (at a mean frequency of 45.1%) and most strains grew to significantly higher levels (with a mean log bacterial concentration of 2.2 CFU/mL) during incubation at 30°C. Significant differences in penetration frequency and yolk multiplication were observed between individual strains and between serotypes (S. Enteritidis > S. Heidelberg for both parameters). Penetration and multiplication were significantly less frequent during incubation at 20°C. These results demonstrate that controlling ambient temperatures during pre-refrigeration storage may be an important adjunct to prompt refrigeration for limiting *Salmonella* growth in eggs and thereby for preventing egg-transmitted human illness.

T4-10 Microbial Levels and Pathogen Prevalence Associated with Restricted Shell Eggs

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Restricted shell eggs not meeting quality standards for retail, but maintaining acceptable quality for inclusion in further processed eggs, are often diverted to further processing. A study was conducted to characterize the microbiological populations present on and in these eggs. On a single day, restricted eggs were collected from three shell egg processing plants a total of three times (replicates). Six shells or egg contents were combined to create a pool. Ten pools of each (shells and contents) were formed for each plant per replicate. Shells and membranes were macerated in sterile phosphate buffered saline. Contents were stomacher blended to form a homogeneous mixture. Total aerobic microorganisms and *Enterobacteriaceae* were enumerated. The prevalence of *Salmonella*, *Campylobacter* and *Listeria* were determined by cultural methods. Average aerobic counts associated with the shells were 4.3 log CFU/ml and with the contents were 2.0 log CFU/ml. There were plant replicate differences for both types of aerobic samples ($P < 0.05$ and $P < 0.01$, respectively). Average *Enterobacteriaceae* level associated with the shell was 2.4 log CFU/ml and less than 0.1 log CFU/ml in the egg contents, with 36.7% of samples being positive. One shell sample (0.5% of total samples) was *Campylobacter* positive. Two shell samples (1.1% of total samples) were *Salmonella* positive. Twenty-one percent of samples were positive for *Listeria* (33 – shells; 5 – contents). While current pasteurization guidelines are based on *Salmonella* lethality, the results of this study indicate a need to revisit the guidelines to determine effectiveness for other pathogenic species.

T4-11 Egg Monitoring Program: Sampling Procedures and *Salmonella* Results

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The Food Safety and Inspection Service (FSIS) develops regulations for egg products under the authority of the Egg Products Inspection Act (EPIA) of

1970. The law requires that all egg products (liquid, frozen and dried) distributed for consumption be pasteurized. Pasteurization destroys *Salmonella* without cooking the eggs, or affecting their color, flavor, or nutritional value. The egg processing plants are responsible for testing pasteurized egg products for *Salmonella*; FSIS Directive 10,230.4 provides information for FSIS personnel about the *Salmonella* surveillance program. The efficacy of this program is checked by the FSIS "*Salmonella* in pasteurized liquid, frozen or dried egg products monitoring programs"; samples are taken and submitted to one of the three FSIS Field Service Laboratories for *Salmonella* analysis. For the period from 1995–2005, 87 positives were detected out of the 17,774 samples tested. These *Salmonella* positive results were distributed over 7 categories: liquid egg whites (24/3,728), liquid whole eggs or yolks with less than 2% added ingredients other than salt or sugar (15/5,126), liquid whole eggs with added yolks or whole egg blends (7/2,119), liquid whole eggs or yolks with more than 2% sugar or salt added (29/3,884), dried yellow egg (11/1,416), spray dried egg whites (1/1,381) and pan dried egg whites (0/120). A time comparison by current year indicates that *Salmonella* positives in most egg product categories have decreased since 1995. *S.* Heidelberg and *S.* Enteritidis were the predominant, followed by *S.* Typhimurium, *S.* Branederup, and *S.* Montevideo.

T5-01 *Listeria monocytogenes* Strains Commonly Isolated from Foods Contain Virulence-attenuating Mutations in *inlA*

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Listeria monocytogenes is a human foodborne pathogen that causes severe invasive disease in susceptible hosts, and the internalins (i.e., *inlA* and *inlB*) play a key role in the pathogenesis of listeriosis. A significant proportion (> 30%) of *L. monocytogenes* strains isolated from foods carry mutations resulting in premature stop codons (PMSCs) in *inlA* and thus production of a truncated and secreted gene product. We created a set of paired isogenic mutants in which (i) *inlA* PMSC mutations were introduced into natural strains carrying a wildtype *inlA* allele, which encoded a full-length gene product and (ii) *inlA* PMSC mutations were reverted to a wildtype *inlA* allele in natural strains that carried an inlA PMSC, to investigate the effect of PMSC mutations in *inlA* on virulence. *inlA*-mediated invasion efficiencies for natural strains and isogenic mutants with PMSCs in *inlA* were significantly lower than those observed for natural strains and revertant mutants carrying a wildtype *inlA* allele in Caco-2 ($P < 0.001$) and HepG2 ($P < 0.0001$) cells. Intragastric guinea pig infections with the paired isogenic mutants showed that *L. monocytogenes* populations recovered from internal organs of animals infected with natural strains and isogenic mutants carrying PMSCs in *inlA* were significantly lower ($P < 0.01$) overall compared to animals infected with natural strains and revertant mutants carrying a wildtype *inlA* allele, supporting the conclusion that PMSC mutations in *inlA* are responsible for virulence attenuation. A laboratory control strain in which a PMSC was introduced in *inlA* showed significantly greater invasiveness in HepG2 cells ($P < 0.05$) as compared to a *DinlAB* null mutant in the same background, and qRT-PCR data demonstrated that *inlB* expression was similar ($P > 0.05$) in the paired isogenic mutants, suggesting that PMSCs in *inlA* do not affect *inlB*. Our data support the conclusion that PMSC mutations in *inlA* are causally associated with attenuated virulence and do not have a polar effect on *inlB*.

T5-02 The Use of Hybridization Techniques as a Tool to Uncover Genomic Factors Associated with the Tropism of *Listeria monocytogenes* for Food, Environmental and Host Niches

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Introduction: *Listeria monocytogenes* is a human foodborne pathogen capable of causing invasive and non-invasive infections. Ubiquitous in the environment, strains are often classified into lineages I (containing serovars 1/2b, 4b, 3b; human illness); II (serovars 1/2a, 1/2c, 3a; food); and III (4a, 4c; food-production). It is not clearly understood how serovars become tropic to a particular niche.

Purpose: The objective of this project is to investigate genomic differences in food, environmental and clinical isolates, and identify specific genomic markers.

Methods: Three different hybridization techniques are being used to elucidate genomic differences in *L. monocytogenes* isolates: mixed genome DNA microarrays, dot-blot hybridizations and suppressive subtractive hybridization (SSH).

Results: Currently, several genomic features have been identified as present, absent or significantly different in food, environmental and clinical isolates, by use of an in-house constructed mixed genome DNA microarray and dot blot hybridization. Sequences that appear as present or absent among genomes tested include homologies to glucarate dehydratases, 23S rRNAs and conserved hypothetical proteins. Some sequences are present in the majority of genomes tested, but array results show a significant difference in hybridization tendencies, suggesting there may be enough sequence diversity to observe a biological effect in the organism. These sequences have homologies to a â-glucosidase, subunit B of a DNA gyrase and cystathionine â-lyase. The function of these sequences will be studied via creation of knockout mutants. While SSH was able to identify genomic differences between *L. innocua* and *L. monocytogenes*, it currently has not been able to identify the differences in *L. monocytogenes* isolates seen in the array results.

Significance: These techniques will provide insight into the mechanisms by which clinical, environmental, and food strains are able to adapt and survive in different niches, and possibly allow for the identification of host and/or bacteria-specific markers required for infection to occur.

T5-03 Contributions of Transcriptional Regulators to *Listeria monocytogenes* Growth at Refrigeration Temperature

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Introduction: Transcriptional regulators are important modulators of bacterial gene expression under environmental stress conditions. As the foodborne pathogen *Listeria monocytogenes* has the ability to grow at refrigeration temperatures, we evaluated the contributions of transcriptional regulators to *L. monocytogenes* growth under this stress.

Purpose: The purpose of this study was to identify transcriptional regulators as potential targets to specifically inhibit *L. monocytogenes* growth at refrigeration temperature.

Methods: *L. monocytogenes* 10403S wild-type strain and isogenic null mutants for 4 alternative sigma factor (Δ*sigB*, Δ*sigH*, Δ*igC*, *and* Δ*sigL*), 2 negative regulators (Δ*ctsR* and Δ*hcA*); and 15 two-component response regulator (TCRR) mutants were grown in Brain Heart Infusion (BHI) broth at 4°C. Growth was measured by spiral plate counts and optical density (OD). Whole-genome microarray experiments were performed on *L. monocytogenes* 10403S to define the cold response regulon and to identify transcriptional regulator-dependent genes differentially expressed at 4°C and 37°C.

Results: Cell enumeration data indicated that Δ*sigH*, Δ*sigC*, Δ*sigL*, Δ*ctsR*, and TCRR mutants Δ*lisR*, Δ*lmo1172*, Δ*lmo1060* have growth defects when compared to wild-type on Day 12 of growth at 4°C ($P < 0.05$), which was also supported by OD data. Microarray analysis revealed 105 and 170 genes upregulated during logarithmic and stationary phase, respectively, at 4°C (as compared to growth at 37°C), while 74 and 102 genes were downregulated during logarithmic and stationary phase at 4°C, respectively (adjusted $P < 0.001$; fold-change 2). Genes upregulated at 4°C during both stationary and log phase included those encoding one TCRR, a cold shock protein, and RNA helicases, whereas genes encoding selected virulence factors and heat shock proteins were downregulated at 4°C.

Significance: Our results indicate that multiple transcriptional regulators contribute to *L. monocytogenes* growth at refrigeration temperature and identify genes differentially expressed in *L. monocytogenes* grown at refrigeration temperature.

T5-04 Microarray Characterization of *Listeria monocytogenes* Genes Regulated by HrcA and CtsR

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Introduction: The foodborne pathogen *Listeria monocytogenes* uses a number of transcriptional regulators, including the negative regulators HrcA and CtsR and positive regulators, such as alternative sigma factors, to control gene expression under different environmental conditions and in response to stress.

Purpose: To define HrcA and CtsR regulons.

Methods: The full genome microarray experiments were conducted to compare transcript levels between (i) a D*ctsR* strain and wild-type (wt) strain 10403S, (ii) the D*ctsR* strain and an *ictsR-mcsA* strain expressing the *ctsR-mscA* operon in trans under control of an IPTG-inducible promoter, and (iii) a D*hcA* strain and the wt strain.

Results: We identified 62 CtsR-dependent genes that showed significant expression ratios (adj. $P < 0.05$), with ≥ 1.5-fold differential expression either between Δ*ctsR* and wt or between Δ*ctsR* and *ictsR-mcsA*. We also identified 61 HrcA-dependent genes that showed significant expression ratios (adj. $P < 0.05$), with ≥ 1.5-fold differential expression between Δ*hcA* and wt. Hidden Markov Model (HMM) searches were used to identify consensus CtsR or HrcA binding sites downstream of or overlapping with a gene's promoter region. Combined with microarray analysis, HMM searches show CtsR and HrcA directly repress at least 10 and 8 genes, respectively. Interestingly, seven genes showed differential expression in both the Δ*ictsR* and Δ*ihcA* strains, even though HMM analyses indicated that only one of these seven genes was directly regulated by HrcA.

Significance: Our data not only define the CtsR and HrcA regulons but also provide preliminary evidence that transcription of some genes may be directly or indirectly regulated by both CtsR and HrcA, which is important for a comprehensive understanding of stress response systems in this pathogen.

T5-05 Sigma B Contributions to Stress Response and Virulence in Select *Listeria monocytogenes* Strains Representing Lineages I, II, IIIA, and IIIB

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Introduction: Multiple phylogenetic analyses of *L. monocytogenes* strains have identified three distinct lineages. Lineages I and II are associated with human listeriosis; lineage III, consisting of subgroups IIIA and IIIB, generally is not. σB, encoded by *sigB*, is an alternative sigma factor previously demonstrated to function in a *L. monocytogenes* lineage II strain as a central regulator of stress response and virulence.

Purpose: The objective of this study was to measure relative contributions of σB to stress response and virulence among strains representing distinct *L. monocytogenes* lineages.

Methods: Δ*sigB* mutants were created in selected wildtype strains representing each *L. monocytogenes* lineage and subgroup. Stress survival phenotypes and invasion capacities of logarithmic- and stationary-phase cells grown in BHI were measured by standard plate counts. Survival of acid or oxidative stresses was characterized by determining numbers of survivors following exposure of wildtype and mutant cultures to pH 2.5 for 60 min or to 13.0 mM cumene hydroperoxide in dimethyl sulfoxide for 15 min. Relative invasiveness was measured using Caco-2 human intestinal epithelial cells.

Results: σB significantly contributed to stress survival and invasiveness of *L. monocytogenes* representatives from lineages I, II, and IIIB ($P < 0.05$). It did not

appreciably contribute to survival and invasiveness of the IIIA representative or of stationary phase F2365, a lineage I food isolate from the 1985 Jalisco cheese outbreak ($P > 0.05$). Furthermore, σB had a limited role in acid and oxidative stress survival and Caco-2 invasion in logarithmic-phase cells.

Significance: These results show that the relative importance of σB in stress response and virulence differs among *L. monocytogenes* strains representing the distinct lineages. Our data predicts a wide range in responses to environmental stress for the full spectrum of *L. monocytogenes* strains.

T5-06 Survival and Subsequent Acid Resistance of Acid Adapted or Osmotically Shocked *Salmonella enterica* Serovar Enteritidis PT 4 and *Listeria monocytogenes* in Traditional Greek Appetizers Stored at 5 and 15°C

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Introduction: Traditional Greek salads (consisting of chopped vegetables, yogurt, olive oil, cheese and/or mayonnaise) are popular appetizers of low pH (3.60–4.43). Despite their intrinsic safety, pathogens have been reported to survive and become acid resistant. "Stress-hardened" pathogens may be more likely to compromise the safety of these products.

Purpose: The purpose of this study was to evaluate the survival of acid adapted or osmotically shocked *Listeria monocytogenes* and *Salmonella enterica* serovar Enteritidis PT4 in various appetizers at chill or ambient temperature, and to assess the acid resistance of surviving populations.

Methods: Cultures of *L. monocytogenes* and *S.* Enteritidis PT4 were prepared as follows: (a) nonadapted in tryptic soy broth (TSB) without glucose, (b) acid adapted in TSB with 1% glucose, (c) osmotically shocked (1 h at NaCl 10–20%). Cultures were inoculated (10^6 CFU/g) in taramasalad (pH 3.60–3.80), tzatziki (pH 4.11–4.18), Russian (pH 3.97–4.17) and cheese (4.32–4.43) salads, and stored at 5 or 15°C for up to 60 days. Acid resistance was evaluated periodically in TSB (pH 1.5 with HCl) at 30°C for 1 h. Survivors and spoilage organisms were enumerated on selective and nonselective media, respectively, and results were compared using ANOVA.

Results: *L. monocytogenes* showed considerably higher survival ($P < 0.05$) than *Salmonella* in all cases. Overall, stress adapted/shocked *L. monocytogenes* survived better ($P < 0.05$) than nonadapted cells, especially at 5°C. Adaptation or shock did not affect ($P > 0.05$), but rather sensitized *Salmonella*. Following exposure to pH 1.5, acid adapted *L. monocytogenes* remained detectable for 33 days in cheese salad at 5°C, while nonadapted cultures died off within 21 days. No such acid resistance was recorded for *Salmonella*.

Significance: Data suggest sufficient endogenous safety of products for *Salmonella*. Conversely, contamination with *L. monocytogenes* subjected to food related stresses may compromise their safety, especially in products of higher pH, such as cheese salad.

T5-07 An Enhanced Discriminatory Scheme for PFGE-based Subtyping of *Salmonella* Enteritidis

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Introduction: *Salmonella* Enteritidis has emerged as one of the most common causes of non-typhoidal salmonellosis in humans. This zoonotic bacterium is often associated with foodborne illness derived from poultry sources, including broiler chickens, eggs, and foods containing eggs. From a molecular epidemiological perspective, the largely uniform genomic structure of *S.* Enteritidis has complicated the use of molecular genetic analysis to discriminate various strains of this pathogen. Conventional PFGE subtyping protocols, capable of differentiating most salmonellae, are largely inadequate for the discrimination of *S.* Enteritidis strains, owing to the highly homogeneous genetic structure of this serovar. Effective strain discrimination, however, is requisite for successful trace back of an outbreak.

Purpose: The purpose of this study was to search an enhanced discriminatory scheme for subtyping *Salmonella* Enteritidis wih PFGE.

Methods: Using a group of highly homogeneous *S.* Enteritidis strains from poultry sources, in a search for increased strain resolution several macro-restriction endonucleases (*SfiI*, *PacI*, and *NotI*) were explored separately and in conjunction with enzymes traditionally applied to *Salmonella* PFGE (i.e., *XbaI*, *BlnI*, and *SpeI*).

Results: A dendrogram derived from the combined analysis of six enzymes was highly discriminatory, with only 38% of strains composing unresolved clusters with an average of 3.6 strains per cluster. Additionally, one three-enzyme combination, SfiI/PacI/NotI, revealed substantial discrimination within the dendrogram, rivaling the six-enzyme tree in diversity. The SfiI/PacI/NotI analysis revealed 47% of strains in unresolved clusters, with only 3.3 strains per cluster. These findings paralleled the number of informative bands yielded by each enzyme with NotI, with the highest level of variation (98%) and the greatest number of unique PFGE pattern types (n = 26).

Significance: In total, these data support a more discriminatory PFGE-based subtyping scheme for *S.* Enteritidis that includes use of several previously unexploited restriction enzymes.

T5-08 Characterization of Integrons, Antimicrobial Resistance Genes and Virulence Genes of *Salmonella enterica* Isolated from Foodstuff and Related Sources

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Multi-resistant *Salmonella* isolates of different serovars are increasingly common worldwide and have been associated with increased morbidity and mortality of humans and animals. The purpose of this

study was to characterize *Salmonella enterica* virulence and antimicrobial determinants in 19 antibiotic multiresistant strains, comprising 9 different serovars, isolated in Brazil. They were submitted to the detection of virulence genes (*invA, aceK, sopB, slyA* and *h-li*), resistence genes (*aadA1, aadB1, qacEŒI-sul1, tetA, tetG, sul1, sul2, flost,* bla_{tem1}), integrons (5'NCS–3'NCS) and the transferability of drug-resistance determinants via conjugation. The most prevalent resistances found were to tetracycline (89%), sulfameth oxazole (79%), ampicillin (63%), chloramphenicol (58%) streptomycin (47%) and nalidixic acid (47%). Ten (52%) of 19 *Salmonella* isolates tested had integrons class I ranging in size from 0.7 to 2.7 kb, which carried several resistance genes including aadA1, *qacEŒI-sul1, tetA, sul1,* bla_{tem1}. Conjugation studies demonstrated that there was no plasmid-mediated transfer of genes. PCR analyses revealed the presence of the virulence genes *invA, aceK, sopB, slyA* in all isolates and the presence of virulence gene h-li in 17 (89%) of them. The present study showed the occurrence of potential virulence genes and that integron-mediated resistance genes contributed to the multiresistance phenotype observed in the isolates. However, several resistance genes were located outside the integron structure, as independent genes. A better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads will enable the design of intervention strategies to reduce its progression. Acknowledgement: FAPESP (05/57779-3; 06/50813-4).

T5-09 DSC Transcription Analysis of *stx*I and *marA* Genes in *Escherichia coli* O157:H7 Treated with Sodium Benzoate

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Introduction: Expression of the multiple antibiotic resistance *(mar)* operon causes increased antimicrobial resistance in bacterial pathogens. The operon activator *MarA*, can alter expression of > 60 genes in *E. coli* K12. However, data are lacking concerning expression of virulence and resistance genes when foodborne pathogens are exposed to antimicrobials.

Purpose: This study was conducted to determine transcription of *marA* (*mar* activator) and *stx*1A (Shiga-like toxin 1) genes of *E. coli* O157:H7 EDL933 grown in the presence of sodium benzoate.

Methods: *E. coli* O157:H7 was grown in Luria-Bertani broth (LB) containing 0 (control) and 1000 ppm sodium benzoate at 37°C for 24 h, and total RNA was extracted. Primers were designed for the housekeeping gene, *hemX* (209 bp), and *marA* (261 bp) gene; previously reported primers were used for *stx*1. Ten-fold dilutions of RNA were used in a one-step reverse-transcriptase-PCR (RT-PCR) reaction, and then visualized using gel electrophoresis to determine transcription levels. All experiments were replicated at least twice.

Results: For *marA* and *stx1*, RT-PCR products were detected at a 1-log greater dilution in the control than in treated cells, although cell numbers for each were similar (7.28 and 7.57 log CFU/ml, respectively). This indicates a greater (albeit slight) level of transcription in the control as compared with treated cells. The *hemX* gene showed the same level of expression in control and treated cells, validating *hemX* as an appropriate housekeeping marker.

Significance: Understanding adaptations of *E. coli* O157:H7 under antimicrobial exposure is essential to better understand and implement methods to inhibit or control survival of this pathogen in foods. These data indicate that *stx*1 and *marA* genes could play a role in survival of pathogens treated with sodium benzoate. Microarray studies are ongoing to gain insight into genes significantly regulated in *E. coli* O157:H7 treated with sodium benzoate.

T5-10 Studies on Biofilm Formation in Enteropathogenic *Escherichia coli*

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Enteropathogenic *Escherichia coli* (EPEC) are a group of diarrheagenic *E. coli* that cause intestinal infection in children, mostly in third world countries, but also in developed countries. Upon excretion from their host, EPEC bacteria face hostile conditions. To overcome these conditions, EPEC, like other bacteria, often produce biofilms. Bacteria within the biofilms generally exist in slime-embedded bacterial communities which confer resistance to numerous environmental stresses and antimicrobial agents. Consequently, biofilms containing foodborne pathogens pose a health hazard when they contaminate agricultural produce and various food processing contact surfaces. The objective of this study was to identify and characterize EPEC genes involved in biofilm formation on abiotic surfaces. We have used two complementary genetic approaches. The first was transposon mutagenesis, and the second was site-directed mutagenesis of genes involved in synthesis of colanic acid (CA) and cellulose, two major capsular constituents of EPEC. Using Tn10-mutagenesis, several mutants were isolated, which exhibited low levels of biofilm formation compared to the wild type (wt) strain. Two of these mutants were further characterized and was found to have an insertion in *csgF*, and *crl*, both involved in curli production and regulation, respectively. Curli are proteinaceous filaments produced on the surface of numerous *Enterobacteriaceae*. The two mutants were deficient in both attachment and biofilm formation capabilities on several inert surfaces, as well as at the liquid-air interface. Complementation experiments with mutants expressing recombinant CsgF and *crl* regained wt phenotype. Site-specific mutations in genes required for CA and cellulose synthesis reveal that cellulose, but not CA, is required for biofilm formation. These observations support a major role for curli and cellulose in EPEC attachment to and biofilm formation on abiotic surfaces.

T5-11 Comparison of Virulence Plasmid (pYV/pCD)-associated Phenotypes in *Yersinia* Species

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Introduction: *Yersinia pestis, Y. enterocolitica,* and *Y. pseudotuberculosis* harbor a virulence plasmid (pYV/pCD: 70-kb) which is essential for infection. In all

three species, carriage of pYV/pCD imparts the calcium-dependent growth phenotype (low calcium response [Lcr]: pin point, size = 0.36 mm), when cultured at 37°C. The pYV/pCD in *Y. enterocolitica* has been correlated with several other phenotypic characteristics including colony morphology/size (size = 1.13 mm), crystal violet (CV) binding (dark-violet colony), Congo red (CR) uptake (red pin point colony, size = 0.36 mm), autoagglutination (AA), and hydrophobicity (HP) which are expressed at 37°C.

Purpose: This study was undertaken to determine whether these phenotypic characteristics of pYV/pCD were expressed in *Y. pestis* and *Y. pseudotuberculosis*.

Methods: The LCR and CR uptake was determined by the appearance of red pinpoint colonies. The colony morphology/size and CV binding was determined by the appearance of small dark-violet colonies. Hydrophobicity was examined for latex particle agglutination. Auto-agglutination was determined with Eagle's minimal essential medium supplemented with 10% fetal bovine serum.

Results: All three species demonstrated LCR, CV binding, and CR-uptake. However, CR-uptake in *Y. pestis* was demonstrated only on calcium-deficient medium, whereas this phenotype was expressed on both calcium-deficient and low-calcium agarose media for *Y. pseudotuberculosis* and *Y. enterocolitica*. These phenotypes were detectable within 24 h at 37°C in *Y. enterocolitica* and *Y. pseudotuberculosis*, but at 48 h in *Y. pestis* due to slower growth rate at 37°C. The colony morphology/size, AA, and HP characteristics were expressed in *Y. pseudotuberculosis* as in *Y. enterocolitica*, but these three phenotypes were not expressed in *Y. pestis*.

Significance: The CR-uptake by *Y. pestis* only in calcium deficient medium and differences in time to express Lcr, CR-uptake, and CV binding provide the means to identify *Y. pestis* colonies in clinical samples, animals, and food.

T5-12 DSC Elongation Factor EF-G as an Indicator of Cell Viability

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To enhance understanding of the processes leading to inactivation of foodborne pathogens in inimical food environments, we assessed the efficacy of quantitative PCR (QPCR) in the analysis of cell viability and activity of *L. monocytogenes*. We measured expression of the tufA gene which codes for the protein elongation factor EF-G. This gene is highly expressed under normal growth conditions and is essential for transcription and translation. Cultures of *L. monocytogenes* ScottA and Fw 03/0035 were held in nutrient broth poised at a_w 0.90 (NaCl) and pH 3.50 and samples periodically withdrawn for targeted tufA gene QPCR and viable count. Although viable cells numbers decreased from 10^8 to less than the detection level (1.3×10^1 cells ml^{-1}), the tufA mRNA level remained stable. To determine whether tufA mRNA was synthesised despite the apparent lack of viable cells or whether levels of this transcript were more stable than anticipated, additional experiments were undertaken. Cells were held under: i) mildly lethal temperature (55°C), ii) presence of rifampin (which inhibits DNA-dependent RNA polymerase) and iii) high temperature and rifampin. While the tufA QPCR signal declined by a factor of 1000, the number of cells that could form colonies declined by $>10^8$. This raises questions about whether *L. monocytogenes* under mildly lethal conditions of pH and a_w or high temperature retain viabilty after being rendered non-culturable. Studies are continuing to assess whether the observed newly synthesised mRNA signals are due to residual cellular activity or whether cells are intact, but non-culturable, and whether such cells can recover the ability to grow.

T6-01 *Listeria monocytogenes* in Fermented Sausages; Effect of Stress Factors

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Introduction: During food processing, bacteria are stressed by factors that are commonly added sequentially and sometimes simultaneously. It has been suggested that if pathogens such as *Listeria monocytogenes* combat the first stress, there is a high probability that they will adapt to this stress and become more resistant to subsequent stresses.

Purpose: The purpose of this study was to evaluate the effects of selected sequential or simultaneous environmental stresses relevant to salami processing (pre-inoculation stresses) on subsequent behavior of *L. monocytogenes* during the storage of sliced salami (post-stressing survival) formulated with modified recipes.

Methods: Stresses including combined acid (lactic acid) and osmotic (NaCl) challenges were applied to *L. monocytogenes* Scott A prior to inoculation onto the surface of sliced fermented sausages formulated with or without the addition of 0.3% oregano plant. Inoculated slices were stored under vacuum at 5 and 15°C for 45 or 27 days, respectively. Cells were recovered on TSAYE, and PALCAM agar. Bacterial survivors were plotted and the decimal reduction times were compared by ANOVA.

Results: Results indicated that survival of *L. monocytogenes* during storage of sausages was highly affected by the pre-inoculation stresses as well as storage conditions. More specifically, during exposure to pre-inoculation stresses the survival of *L. monocytogenes* was greatly affected by the rate and the sequence at which pH and a_w was reduced. Although the rate at which the numbers of *L. monocytogenes* decreased below detectable limit was faster at 15°C when compared to storage at 5°C, bacterial populations remained detectable by the end of storage period at both storage temperatures.

Significance: These results clearly indicate that extended survival of *L. monocytogenes* during storage is strongly associated with the induction of resistance systems that may remain active for prolonged periods of storage.

T6-02 Inhibitory Effects of Nitrite and Lactate/Diacetate on Survival, Growth and Germination of *Clostridium perfringens* in Cooked Meat and Poultry Products under Abusive Chilling Conditions

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Introduction: The USDA cooling regulations are designed to safely preclude the growth of potentially surviving spores of *Clostridium perfringens* during cooling of cooked meat and poultry products. In some instances involving large diameter products, compliance with these regulations has been difficult to attain.

Purpose: The purpose of this study was to deterine if addition of a lactate/diacetate mixture (Purasal S Optiform PD 4, Purac) to cured meat and poultry products could extend the safe cooling times for large diameter products.

Methods: Six ATCC and NCTC strains of *Clostridium perfringens* were grown in Fluid Thioglycollate and modified Duncan Strong Sporulation Medium. A mixed inoculum of these strains, plated anaerobically onto SFP agar, contained 1.2×10^9 vegetative cells and 4.6×10^4 spores. The mixed inoculum was added to uncured, cured, and cured plus Purasal, raw formulated turkey and beef rolls. Inoculated products were stuffed into 12" diameter fibrous casings and cooked to internal temperatures of 160°F and 155°F respectively. Pieces of each cooked product were then placed in an air cooler (35°F) or held at room temperature (72°F) for 3 days. Samples of raw, cooked and cooled products were analyzed for *Clostridium perfringens* on SFP agar.

Results: No growth of inoculated *C. perfringens* occurred in products chilled in the 35°F cooler, which attained 45°F within 15.5 h. Both uncured products showed rapid growth (~ 5+ log) after 20 h at 72°F. Both cured products showed ~a 1 log increase after 20 h at 72°F with growth reaching 4+ log after 42 h. The products containing nitrite and the lactate/diacetate mixture showed no growth after 72 h at 72°F.

Significance: These data suggest that the USDA cooling requirements could be safely extended for meat and poultry products containing both nitrite and lactate/diacetate.

T6-03 DSC Ultraviolet Light and Dimethyl Dicarbonate to Reduce *Listeria monocytogenes* in Chill Brine

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Chill brines used during Ready-to-Eat meat processing may be an important source of post-processing contamination by *Listeria monocytogenes*. The purpose of this study was to determine the efficacy of ultraviolet light (UV) in combination with dimethyl dicarbonate (DMDC) to reduce *L. monocytogenes* in chill brine. Fresh brine (8.0 % w/w NaCl) was inoculated with a *L. monocytogenes* cocktail (~ 6 log CFU/ml), DMDC (250 or 500 ppm) was added, and the solution was exposed to UV in an Ultraviolet Water Treatment Unit (Model: AMD 150B/1/2T D; Aquionics Inc., Peak output: 254 nm) fitted with an inline chiller to maintain brine temperature of -1°C. Samples were withdrawn at regular intervals for 120 min. When *L. monocytogenes* was no longer detectable via direct plating on MOX, enrichment was performed and suspect colonies were confirmed using API-*Listeria*. When treated with UV alone, *L. monocytogenes* populations decreased from ~6 log CFU/ml to below the detection limit (1 log CFU/ml) in 5 min. However, *L. monocytogenes* was detectable by enrichment though 120 min. When treated with 250 and 500 ppm DMDC alone (i.e., no UV), populations decreased to below detection limit in 90 and 75 min, respectively, but z were detectable by enrichment though 120 min. When treated with UV and 250 ppm DMDC, *L. monocytogenes* decreased to the detection limit in 15 min and was not detected by enrichment after 75 min, whereas 500 ppm DMDC reduced *L. monocytogenes* to the detection limit in 5 min and was not detected by enrichment after 45 min. The results of this work indicate that combinations of UV and DMDC may be more effective than either treatment alone for the reduction of *L. monocytogenes* in chill brines.

T6-04 Effect of Marination and Tenderization Ingredients on Thermal Inactivation of *Escherichia coli* O157:H7 in Beef

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Introduction: Internalization of *Escherichia coli* O157:H7 in non-intact beef products during mechanical tenderization is of concern if such products are undercooked. Evaluation of thermal destruction of the pathogen in beef products formulated with ingredients used in such processes, or having the potential to enhance thermal inactivation, is essential.

Purpose: The objective of this study was to test organic acids (0.2%, wt/wt citric acid; 0.3%, wt/wt acetic acid), potassium and calcium salts (1.8%, wt/wt potassium lactate; 0.63, wt/wt calcium lactate; 0.86%, wt/wt calcium ascorbate; 0.23%, wt/wt calcium chloride), and sodium chloride (2.5%, wt/wt) for their influence on thermal destruction of *E. coli* O157:H7 in a ground beef model. Methods: Batches (700 g) of ground beef (5% fat) were mixed with equal volumes (21 ml) of each treatment-solution or distilled water. Portions (30 g) of the treated ground beef were extruded in test tubes (2.5 x 10 cm). A five-strain cocktail of *E. coli* O157:H7 was introduced at the center of the sample with a pipette. After overnight storage (4°C), simulating product marination, the samples were heated to 60 or 65°C internal temperature in a circulating water bath, and analyzed by plating on tryptic soy agar, modified eosin methylene blue agar, and modified sorbitol MacConkey agar. Two replicates, with sampling in triplicate, were performed.

Results: Treatments with citric and acetic acid showed greater ($P < 0.05$) reduction (4–5 log CFU g^{-1}) of *E. coli* O157:H7, compared to almost all the other ingredients and the control (3–4 log CFU g^{-1}). Addition of sodium chloride maintained the pH (5.54) of the product, reduced weight-losses (16–18%; compared to 20–27% by citric or acetic acid), and resulted

in a 4-log reduction in the pathogen counts during heating.

Significance: Inclusion of ingredients such as citric or acetic acid may improve thermal inactivation of *E. coli* O157:H7 internalized in non-intact beef products, while addition of sodium chloride may reduce cooking losses in such products.

T6-05 Efficacy of Surface Spray Application of Lauric Arginate, Octanoic Acid, and Colicin E1 Prior to Packaging to Control Growth of *Listeria innocua* on Ready-to-Eat Meat Products

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The objective of this study was to evaluate efficacy of a surface spray application of lauric arginate (LAE-10%), octanoic acid (OAC-1%) and Colicin E1 (COL) prior to packaging, and determine their ability to inhibit and control outgrowth of *Listeria* spp. in deli-style hams, roast beef rounds, and turkey breasts. Application of antimicrobials was based on product surface area (in^2) and none of the meat products contained antimicrobials as part of their formulation. Effects of treatments were evaluated thoughout the product shelf life (120 days) and stored at 37°F. Sensory evaluation studies were performed only on USDA-approved food antimicrobials (LAE and OAC). LAE treatment significantly reduced ($P < .05$) initial *Listeria* populations in hams and turkey breasts by an average 1.38 and 1.46-log CFU/cm^2, respectively, compared to controls. Reductions by OAC treatments were also significantly reduced ($P < .05$) for hams and turkey breasts by an average 0.68 and 1.07-log CFU/cm^2, respectively. LAE was the only antimicrobial that maintained reduced numbers of Li on roast beef samples with an average log reduction of 1.28-log CFU/cm^2. Initial log reductions on all RTE products treated with COL were significantly lower ($P < .05$) compared to LAE and OAC treatments. Results from the sensory evaluation indicated some minor off-note flavors but were not significant in turkey samples with OAC, and roast beef and ham samples with LAE. These data indicated that the surface spray application of food antimicrobials prior to packaging can significantly reduce levels of Li on Ready-to-Eat meat products.

T6-06 DSC Pre-harvest Carriage and Diversity of *Escherichia coli* O157:H7 in Feedlot Cattle

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While cattle are a major reservoir of *Escherichia coli* O157:H7, the ecology and dissemination of this organism in slaughter-ready cattle is not fully understood. A study was conducted to investigate *E. coli* O157:H7 prevalence, colonization and diversity in feedlot cattle during the final 120 d of the finishing period. Rectal fecal grab samples were collected from targeted animals representing a sample population of 788 steers every 20–22 days for a total of six sample collections. Fecal samples were analyzed by immunomagnetic bead separation to detect and isolate *E. coli* O157:H7 and presumptive colonies were confirmed using a multiplex PCR assay, which screened for gene fragments unique to *E. coli* O157:H7 (rfbE and flich7) and other key virulence genes (eaeA, *stx*-I, and *stx*-II). Initially, 48.6% of the 788 animals shed *E. coli* containing at least one virulence gene, while 39.8% shed *E. coli* O157:H7 carrying all five genes. Animals were classified as a persistent shedder (PS), transient shedder (TS) or non-shedder (NS), if they consecutively, intermittently or never shed *E. coli* O157:H7, respectively, thoughout the sampling period. Overall, 1.0% and 1.4% of the sample population were classified as PS and NS animals, respectively. All isolates from PS and selected isolates from TS animals were characterized by pulsed field gel electrophoresis (PFGE) typing, which yielded 34 unique subtypes. Only five subtypes overlapped between PS and TS animals, whereas 19 and 10 subtypes were unique to PS and TS animals, respectively. A single predominant subtype represented 50.7% of the 138 isolates characterized, and isolates representing this subtype demonstrated enhanced attachment efficiency in Caco-2 intestinal epithelial cells. Our data show that a small subpopulation of feedlot cattle may become persistently colonized with multiple *E. coli* O157:H7 subtypes and support a relationship between *E. coli* O157:H7 strain-specific attachment efficiency and persistence in the gastrointestinal tract.

T6-07 Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Retail Meat: National Antimicrobial Resistance Monitoring System (NARMS): 2002–2005

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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 humans 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 of 15,524 meats, including chicken breast (n = 3877), ground turkey (n = 3859), ground beef (n = 3904) and pork chops (n = 3884) collected during 2002–2005 in NARMS retail meat surveillance program. Isolates were analyzed for antimicrobial susceptibility and by DNA fingerprinting using PFGE.

Results: A total of 1,042 *Salmonella* isolates were recovered; 513 (49%) were from ground turkey, 453 (43%) from chicken breast, 41 (4%) from ground beef and 35 (3%) from pork chop. The top five serotypes were S. Heidelberg (n = 230), Kentucky (n = 141),

Typhimurium (n = 127, including Typhimurium var. Copenhagen and S.I 4,5,12:i:-), Saint Paul (n = 92), and Hadar (n = 65). Six hundred thirty-six (61%) of the isolates displayed resistance to > 1 of the 15 antimicrobials tested, whereas 151 (14.5%) were resistant to >5 antimicrobials. Three (0.3%) isolates recovered from ground turkey exhibited resistance to >9 antimicrobials. Resistance was most often observed to tetracycline (44%), streptomycin (35%), sulfamethoxazole (26%), and ampicillin (26%), and to a lesser extent, to amoxicillin-clavulanic acid (15%), kanamycin (14%), gentamicin (14%), ceftiofur (13%), and chloramphenicol (3%). 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 4-year period.

Significance: Results indicate that antimicrobial-resistant *Salmonella* isolates are present in retail meats, in particular poultry products, and stress the need for sustained surveillance of foodborne pathogens in retail foods.

T6-08 DSC Characterization of *Campylobacter jejuni* Strains Isolated from Commercial Broilers in Puerto Rico by Pulsed-field Gel Electrophoresis, Multi-locus Sequence Typing and Cytotoxicity Assays

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Introduction: *Campylobacter jejuni* is a frequently identified foodborne pathogen. Clinical presentations are mostly sporadic cases of diarrheal disease, although serious sequela, such as the development of Guillain-Barre syndrome, can also occur. Little is known about the characteristics of *C. jejuni* isolated from commercial broiler chickens in Puerto Rico.

Purpose: We analyzed thirty-one *C. jejuni* isolates obtained from 17 different commercial broiler farms in Puerto Rico by multiplex PCR, E-Test, pulsed field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and cytotoxicity assays on mammalian cell lines.

Methods: Species identification was achieved with a multiplex PCR. E-Test was performed according to manufacturer's guidelines. PFGE and MLST were performed following standard protocols, and percentage of lactate dehydrogenase release (LDR) was measured in Caco-2, Hep-2 and CHO cell lines.

Results: All strains were resistant to vancomycin and trimethoprim, but only one strain was resistant to ciprofloxacin. The resistance to other antimicrobials appears to correlate with previous reports from other geographical regions. The use of two restriction enzymes, *SmaI* and *KpnI*, resulted in the most diverse PFGE patterns. Several strains with identical MLST clonal complexes had different PFGE profiles. One strain possessing unique PFGE profiles with all restriction enzymes belonged to the sequence type (ST) 460, which has been linked to human campylobacteriosis. Other strains belonged to ST-429 and some to a new allelic profile similar to ST-52. A large variability was found with the cytotoxicity assays in mammalian cells. Caco-2 cells showed the lowest values for LDR, while CHO showed the highest values.

Significance: The incidence of ciprofloxacin-resistant among *C. jejuni* strains isolated from commercial broiler chickens in Puerto Rico may be low. For short term epidemiological studies, PFGE is still the technique of choice, and the use of enzyme combinations may be more appropriate. Overall, these strains have unique features, which may affect their pathogenicity capabilities.

T6-09 DSC Logistic Processing of Commercial Broiler Flocks to Reduce Cross Contamination by *Campylobacter*

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Introduction: There is considerable interest in reducing *Campylobacter* contamination of broiler carcasses. An important source of carcass contamination during slaughter is the intestinal content of previously processed *Campylobacter*-positive flocks. Therefore, a logistic schedule in which *Campylobacter*-free flocks are processed first may help obtain carcasses with low contamination after processing.

Purpose: To determine the colonization status of the flocks a week before slaughter and to develop a scheduling system where flocks with the lowest *Campylobacter* contamination are processed at the start of the processing day to avoid cross contamination.

Methods: Ten fecal samples per flock per farm, for a total of three farms per trip, were collected in seven independent trips. After *Campylobacter* enumeration from the samples, flocks were classified as negative or contaminated and were sent for processing within eight days after sampling. A scheduling in which negative flocks were processed first to avoid cross contamination, or contaminated flocks where processed first to induce cross contamination, was used in different days. At processing, each flock was sampled for *Campylobacter* enumeration before and after the chiller. Pulsed-field gel electrophoresis (PFGE) was performed on strains isolated from farms and processing plants to determine DNA relatedness.

Results: Our data suggest that *Campylobacter*-negative flocks processed after *Campylobacter*-positive flocks became contaminated with strains originating from previously processed *Campylobacter*-positive flocks. Isolates with similar PFGE from *Campylobacter*-negative flocks at the processing plant and from *Campylobacter*-positive flocks at the farm confirmed these results. When birds from *Campylobacter*-negative farms were processed first, the carcasses remained

negative thoughout processing. The positive birds that were processed following *Campylobacter*-negative flocks resulted in carcasses with a lower level of *Campylobacter* contamination.

Significance: A scheduling system could avoid cross contamination at the processing plant by processing negative flocks first. This type of logistic processing may also help reduce the number of *Campylobacter* counts in positive flocks during processing.

T6-10 DSC Fate of *Listeria monocytogenes* on Frankfurters at Different Stages from Manufacturing to Consumption

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Introduction: Recommendations by the United States Dept. of Agriculture Food Safety and Inspection Service (USDA-FSIS) indicate that frankfurters may be safely stored at home at 4°C for a week in open and two weeks in vacuum-sealed packages. The Food and Drug Administration (FDA) Food Code recommends that retail and food service operations do not store Ready-to-Eat foods that are not vacuum-sealed for longer than 4 days at 7°C.

Purpose: This study was conducted to validate these recommendations by determining the fate of *Listeria monocytogenes* on inoculated frankfurters under various conditions.

Methods: Frankfurters (two replicates, three samples per treatment) formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) were inoculated (1.79 ± 0.08 log CFU/cm^2) with a 10-strain composite of *L. monocytogenes*. Frankfurters were then vacuum-sealed and stored at 4°C for 60 days after exposure to conditions simulating transportation from a plant to retail stores. At 0, 20, 40, and 60 days, packages were exposed to conditions simulating delivery from stores to consumer homes, opened or held vacuum-sealed at 4 or 7°C for 14 days, and periodically analyzed for pathogen (PALCAM agar) and total microbial counts (tryptic soy agar with 0.6% yeast extract).

Results: Under the conditions tested, pathogen counts remained relatively constant in samples formulated with PL/SD, but in product without antimicrobials reached 7.0 log CFU/cm^2 within 60 days of vacuum-sealed storage (4°C). At day 4 of home storage, pathogen counts increased by less than 1.0 log CFU/cm^2 on frankfurters formulated without PL/SD, regardless of storage conditions and product history. Fastest growth under USDA-FSIS recommended storage conditions (4°C) occurred in samples stored for 20 days prior to home storage; the increases were 2.19 ± 0.8 and 2.40 ± 0.2 log CFU/cm^2 for samples stored for 7 (open packages) and 14 (vacuum-sealed packages) days, respectively.

Significance: It may be advisable to re-evaluate the USDA-FSIS safe storage time limits for frankfurters without antimicrobials in the formulation.

T6-11 Effect of Rinse Volume and Sample Time on Recovery of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterobacteriaceae* from Post-chill Chicken Carcasses

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Introduction: The FSIS HACCP and baseline microbiological sampling of chickens uses a 400 mL whole carcass rinse. Other studies suggest that smaller rinse volumes are equal to or more efficient than the 400 mL rinse. Pick-up time for overnight shipping requires day-shift sampling, which FSIS is concerned could introduce bias into the test results.

Purpose: Study purpose was to determine if time of sampling within a two-shift processing day and rinse volume affected the qualitative recovery of *Salmonella* and the enumeration of *Campylobacter*, *E. coli*, and *Enterobacteriaceae* from processed chicken carcasses.

Methods: Carcass rinse samples (300) were collected over three two-shift processing days from a commercial plant. On each of the days, 50 pairs of carcasses were rinsed with either 100 mL or 400 mL buffered peptone (BP) water and qualitatively sampled for *Salmonella* and quantitatively sampled for *Campylobacter* using standard cultural procedures. *E. coli* and *Enterobacteriaceae* counts were obtained with Petrifilm.

Results: *Salmonella* recovered (26% of carcasses) was not significantly affected by rinse volume, processing shift or farm chickens were grown on, but was significantly affected by house within the farm. *Campylobacter* recovery was low, but the number recovered was higher (Log 0.7 / mL) with a 100 mL rinse than with a 400 mL rinse (Log 0.1/mL). More *Enterobacteriaceae* and *E. coli* were recovered with a 400 mL rinse (Log 1.63 and 1.09) than with a 100 mL rinse (Log 1.12 and 0.73). Neither *E. coli* nor *Enterobacteriaceae* is a good indicator of *Salmonella* or *Campylobacter* on an individual carcass.

Significance: Time of day did not affect recovery of *Salmonella* or *Campylobacter*. Recovery of *Salmonella* was numerically but not significantly higher from 400 mL rinses, but *Campylobacter* recovery was higher from 100 mL rinses. The house that birds were grown in significantly affected microbiological quality of the processed carcasses.

T6-12 DSC An Exploratory Study of Food-handling Practices at Church Dinners in Canada

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Introduction: Community gatherings around food awaken nostalgic feelings of the rural past – times when an entire town would get together monthly, eat, enjoy company and work together. Despite this sense of kinship, there have been at least 37 reported outbreaks of foodborne illness associated with home-cooked products and community dinners in North America since 1973. Informal event infrastructures coupled with recent political pressure for deregulation provides a climate for potential food safety problems.

Purpose: This observational and demographically-based research was undertaken to assess whether a segment of these church food-centered gatherings was inherently safer or more dangerous than other food establishments. This exploratory research was intended to provide a baseline of practices and profiles of individuals responsible for organizing and preparing food at these functions, to be used in future program development.

Methods: A convenience sample of three churches was used for data collection. Profiles of three church-sponsored food events were created using case study methodology. Both quantitative (a questionnaire and observation of preparation practices focusing on foodborne illness risk-factors) and qualitative (attitudes and intentions surrounding safe food preparation acquired though in-depth interviews) methods were used.

Results: Food preparation was completed by volunteers with no formal food safety training who did not always practice foodborne illness risk-reduction procedures. Results included observed violations of temperature-related infractions and adherence to food preparation guidelines (including dish washing procedures, hygiene and sanitation). Home food preparation was common in all three church functions.

Significance: A focus on efficiency and timing of food service was seen, not a focus on microbial food safety. This lack of attention to potential risks and the infrastructure of the events indicate that communication materials and management strategies acquired though specialized formal training may be useful in creating a culture of food safety in church kitchens.

T7-01 Fresh Produce Outbreaks: Using Outbreak Data to Determine Attribution in Developing Risk Assessments

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Recent studies and media reports have highlighted an increase in foodborne illness associated with produce. In 2002, the Center for Science in the Public Interest suggested that poor farming practices might be largely responsible for this increase. In September 2006, pre-packaged spinach contaminated at the farm was associated with an *E. coli* O157:H7 outbreak resulting in at least three deaths and 204 illnesses. However, outbreak data and scientific studies suggest post-harvest contamination due to food handling and preparation may still be the largest risk factor. The purpose of this study was to analyze detailed outbreak data associated with foods of plant origin, to provide more accurate risk factors to better inform priority setting and resource allocation by government risk assessors. Information concerning over 2,300 outbreaks and 800 recalls was collected and analyzed from North American and international sources. For over a decade, approximately 10% of foodborne outbreaks annually were attributed to produce; however, in 2004 that increased to 18.4%. Meat-associated outbreaks have remained consistent at approximately 20% annually. Produce-related outbreaks were divided between bacterial (49.6%) and viral agents (44.6%) while the majority of meat-related outbreaks were associated with bacterial agents (83.4%). Cases associated with produce outbreaks are approximately double those associated with meat. The primary setting for produce outbreaks was in food service (52%), while 30% of meat outbreaks were associated with the home and only 25% with food service. Determining the point of contamination is significant because the most effective control measures should be targeted toward reducing contamination at that point. The large number of outbreaks occurring at food service and of viral origin suggests that produce outbreaks are associated with errors in food handling and preparation. Although Good Agricultural Practices on farm are important, they will not prevent illness from post-harvest cross contamination, particularly in food-service settings or homes.

T7-02 An Evaluation of Risk Management in the New Product Development Process within Food Manufacturers

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As a result of the escalating demand for new food products, there is increasing pressure on processors to be innovative in new product development (NPD). There is, however, a requirement for all companies to assure product safety though effective risk management during the design development process. The purpose of this research was to analyse methods employed by food processors to assure safety during NPD and product re-formulation processes. Managers at 36 food processors were interviewed by use of a structured interview of 10 questions. Of the companies visited, 6 were large processors and 30 were Small Medium Sized Enterprises (SME = less than 250 employees). One-hundred percent of the large companies had a new product development protocol encompassing risk assessment, including HACCP, shelf-life analysis and organoleptic end of life assessment. Only 50% of SME's had a similar NPD procedure. Of the 15 SME companies employing such protocols, 80% (12 companies, i.e., 40% of the total SMEs) had gained 3rd party accreditation from GFSI recognized certification bodies (e.g., BRC standard). Twenty companies stated that the NPD process was driven by customers, and 16% of companies admitted to launching products that had not been subjected to risk assessment or shelf-life analysis. Within the last 5 years, 5% claimed to have recalled products because of shelf-life issues. Protocols surrounding recipe re-formulation were also analyzed. It was found that 61% of the respondents had undertaken product re-formulation, with 45% of these companies undertaking a full HACCP analysis and 64% assessing shelf life through laboratory analysis. A case study of one re-formulation and subsequent product recall within the respondents will be outlined in the presentation. Further results of this study will be discussed in the context of generic recommendations for training strategies for companies to develop NPD risk assessment programs.

T7-03 Developing an Integrated Enteric Disease Surveillance System for Canada

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Integrated surveillance and source attribution have been advocated in Canada and abroad to reduce the burden of enteric diseases. In Canada, C-EnterNet, funded by Agriculture and Agri-Food Canada, and facilitated by the Public Health Agency of Canada, is a new multi-partner, integrated, sentinel site surveillance initiative. The C-EnterNet surveillance system, for enteric pathogens both in the human population and in various exposure sources, was officially launched in its first (pilot) sentinel site, in June 2005, in the region of Waterloo, Ontario (a community of approximately 500,000 residents in an urban/rural area of southwestern Ontario). Active monitoring of enteric pathogens on farms (swine and dairy manure), retail food (raw chicken breasts, pork chops, and ground beef) and untreated surface water was implemented. In parallel, enhanced epidemiological and microbiological data were collected for the human cases, based on a strong collaboration with the local public health unit and both private and public health diagnostic laboratories. In addition to C-EnterNet's ongoing surveillance activities, several episodic research projects were initiated. This presentation will outline the steps taken in the development of this new integrated surveillance system for Canada. In addition, the challenges and lessons learned during the first year of operation at the pilot site will be shared with recommendations for the future of this integrated surveillance system.

T7-04 Developing Risk Profiles to Assist Regulatory Decision Making

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FDA's Center for Food Safety and Applied Nutrition (CFSAN) continues to seek new risk-based ways to describe food safety issues and assist in regulatory decision-making. One new approach is in the development and application of risk profiles. The term risk profile (RP) has been defined by the Codex Commission for Food Hygiene as a decision tool which it uses to consider proposed new work in microbiological risk assessment (MRA)/risk management (RM). CFSAN has adapted this tool for the purpose of describing a food safety problem by identifying those elements of the hazard or risk that are relevant to a food safety issue and potential RM options. Consideration of the information given in the RP may result in a range of initial decisions, such as commissioning a MRA, request for gathering additional information or implementing an immediate and/or provisional decision. In some cases, no further action may be needed. Where the conduct of a risk assessment is one of the RM options, the RP is a means for establishing the risk assessment policy and scope of the risk assessment charge. CFSAN is developing four RPs: hepatitis A virus in fresh produce; transmission of norovirus from food workers to ready-to-eat food; *Listeria monocytogenes* in fresh produce and fresh-cut products, and raw milk cheeses. The decision was based on a process that balances the Center's regulatory needs with an evaluation of the feasibility of conducting a risk assessment, and available resources. A key component of developing a RP is communication between the RP teams and the risk managers to determine the scope of the RP and ensure the RP addresses the risk management questions. The development of RPs at CFSAN will be illustrated in the presentation.

T7-05 Predictive Model for the Growth and Survival of *Vibrio vulnificus* in Chesapeake Bay Shellstock Oysters

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Vibrio vulnificus is the leading cause of reported human death in the US associated with the consumption of shellfish, especially oysters. Consumption of contaminated raw oysters can lead to death within 24 h in susceptible individuals. The growth and survival of *V. vulnificus* in post-harvest oysters over a narrow range of storage temperatures have been studied. Predictive models for environmental levels of *V. vulnificus* as a function of seawater salinity and temperature were also reported. However, predictive models have not been reported for the growth and survival of *V. vulnificus* in post-harvest shellstock oysters stored at temperatures relevant to commercial and consumer handling practices. The objective of this study was to address this data gap. In this study, oysters were collected from the Chesapeake Bay from June though August 2006 and then stored at 5, 10, 20, 25, and 30°C for selected time intervals. At each test interval, two separate samples of six oysters each were analyzed by a direct plating method, using an alkaline phosphatase-labeled DNA probe targeting the species-specific *V. vulnificus* hemolysin (vvhA) gene. The Baranyi D-model was fitted to the *V. vulnificus* growth and survival data to define the parameters of lag phase duration (LPD) and growth/inactivation rate (GR). *V. vulnificus* was slowly inactivated at 5 and 10°C and GR of -0.008 and -0.007 log CFU/h, respectively. The maximum GR (0.029 log CFU/h) was observed at 30°C. At 15, 20 and 25°C, the GR was 0.005, 0.010, and 0.021 log CFU/h, respectively. A LPD was only observed at 10°C (218 h). Secondary model for GR had bias and accuracy factors of 1.00 and 1.00, respectively. This model will provide valuable tools for designing and implementing food safety systems and assessing the impact of *V. vulnificus* disease.

T7-06 Modeling Transfer of *Listeria monocytogenes* between Meat Slicer and Ham during Slicing

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Listeria monocytogenes has been implicated in several listeriosis outbreaks linked to the consumption of pre-sliced Ready-to-Eat (RTE) deli meats. The possible contamination of sliced RTE meats by *L. monocytogenes* during the slicing process has become a public health concern. The objectives of this study were to investigate the transfer of *L. monocytogenes* between a meat slicer and ham slices, and to develop empirical models to describe the transfer during slicing. A six-strain cocktail of *L. monocytogenes* was inoculated onto a slicer blade to an initial level of ca. 3, 6, or 9 log CFU/blade, and then the ham was sliced to a thickness of 1–2 mm (Case I). As a second (Case II) cross-contamination scenario, a clean blade was used to slice ham that was previously inoculated with *L. monocytogenes*, followed by slicing ham that was not inoculated. The slicing rate was maintained at 3 slices/min for both cases. Although the recovery ratio was less than 100%, more ham slices were contaminated with *L. monocytogenes* when the blade was contaminated with higher initial levels of *L. monocytogenes*. For Case I, the number of contaminated slices compared to the total number of slices collected was 5/40, 30/40 and 150/150 for inoculation levels of 3, 6, and 9 log CFU/blade, respectively. *L. monocytogenes* stayed on the blade longer and contaminated more ham slices for Case II than for Case I, results for Case II being 5/40, 110/120, and 150/150 for inoculation levels of 3, 6 or 9 log CFU/test, respectively. The microbial count on the 150th consecutive slice, starting from an initial level of 9 log CFU, was about 1.0 log CFU, higher for Case II than for Case I. The empirical models developed well predicted the trend of *Listeria* transfer between the blade and ham slices.

T7-07 Modeling the Survival of *Escherichia coli* O157:H7 during Fermentation, Drying, and Storage of a Soudjouk-style Fermented Dry or Semi-dry Sausage

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Cases of foodborne illness have been linked to the consumption of fermented dry or semi-dry sausages (FDSS) contaminated with *Escherichia coli* O157:H7. The purpose of this study was to model the inactivation of *E. coli* O157:H7 during FDSS manufacturing and storage. Beef batter (20% fat) containing 0.25, 0.5, or 0.6% dextrose, spices and a lactic acid starter culture was inoculated with a three-strain cocktail of *E. coli* O157:H7 to approximately 7.0 log CFU/g. The raw sausage (pH 6.0, a_w 0.97) was fermented at 24°C with RH 90–95% for 72 h to pH 5.2, 4.9, or 4.6, and then dried at 22°C with 80–85% RH to a_w 0.92, 0.89, or 0.86. The sausages were vacuum-packed and stored at 4, 21, or 30°C for up to 60 days. During fermentation, the inactivation of *E. coli* O157:H7 could be described by two linear regressions in which the inactivation rates were 0.0224 and 0.1333 log CFU/g for every 0.1 unit decrease in pH in the range of pH 5.55–4.82 and pH 4.81–4.50, respectively. During drying, the inactivation could be described by a linear regression in which the inactivation rates were 0.3167, 0.4833, and 0.5833 log CFU/g for every 0.01 unit decrease in a_w for sausages fermented to pH 5.2, 4.9, and 4.6, respectively. The inactivation rates of *E. coli* O157:H7 (log CFU/day) during storage at 4, 21, or 30°C were fitted to a quadratic model as a function of sausage pH and a_w and storage temperature. The model indicated that the inactivation during storage was significantly higher ($P < 0.01$) in sausages with lower pH and lower a_w that were stored at 30°C. These models will enable manufacturers of FDSS to estimate the reduction of *E. coli* O157:H7 for their products and modify the product formulations and processes to meet existing food safety guidelines.

T7-08 Modeling to Predict the Fate of *Listeria monocytogenes* on Commercial Frankfurters as a Function of Storage Temperature and Time

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Introduction: Mathematical modeling may be useful in predicting the fate of *Listeria monocytogenes* in Ready-to-Eat meat products under various conditions.

Purpose: The objective of this study was to develop models to predict *L. monocytogenes* populations in frankfurters as a function of storage temperature and time.

Methods: *L. monocytogenes* (10-strain composite) was inoculated (1–2 log CFU/cm^2) on commercial beef or turkey frankfurters, which were formulated without (WO) or with antimicrobials (WA; 1.5% potassium lactate plus 0.05% sodium diacetate). Pairs of frankfurters were stored in vacuum bags at 4, 7, or 12°C, and analyzed periodically up to a 90-day storage period, depending on product and temperature. Total bacterial and *L. monocytogenes* populations were enumerated on tryptic soy agar plus 0.6% yeast extract and PALCAM agar, respectively. *L. monocytogenes* data were fitted with the Baranyi model to calculate lag phase (day) and rates (death or exponential growth rate; log CFU/cm^2/day) for each product and temperature; rates and lag phases were then fitted with a polynomial equation as a function of temperature (two replications, three samples each).

Results: Rates were higher ($P < 0.05$) at 12°C [WA: 0.18 ± 0.05 (beef), 0.21 ± 0.13 (turkey); WO: 0.57 ± 0.04 (beef), 0.38 ± 0.24 (turkey)] than at 4°C [WA: -0.01 ± 0.00 (beef), 0.03 ± 0.05 (turkey); WO: 0.18 ± 0.00 (beef), 0.09 ± 0.13 (turkey)] and 7°C [WA: 0.07 ± 0.10 (beef), 0.08 ± 0.11 (turkey); WO: 0.18 ± 0.08 (beef), 0.29 ± 0.02 (turkey)]. Lag phases were 0–72.9 days depending on the product and temperature. After the rates and lag phases were fitted with a polynomial equation as a function of temperature, predicted rates and lag phases were 0.00–0.21 (WA) and 0.09–0.57 (WO), and 0–40.8 (WA) and 0.5–6.4

(WO), respectively, for beef and turkey frankfurters combined. Models predicting the fate of *L. monocytogenes* were then developed using the predicted values as a function of storage temperature and day.

Significance: The models developed may be useful to select conditions for control of *L. monocytogenes* populations on frankfurters, and may be applicable in the development of models for other products, conditions or pathogens.

T8-01 *Salmonella* Newport as Reported by the Animal Arm of the National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS)

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Introduction: 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 resistance is trending upward. Of particular concern starting in 2000, was the emergence of multiple drug resistant (MDR) *Salmonella* Newport.

Purpose: The purpose of this study was to determine the trend of *S.* Newport submitted to the animal arm of NARMS for genotypic and phenotypic characterization from 1997 though 2005.

Methods: Isolates submitted to NARMS were tested for susceptibility to 16 antimicrobials using a custom made panel and a semi-automated broth microdilution system (Sensititre, Trek Diagnostics, Cleveland, OH). Isolates were further characterized by pulsed field gel electrophoresis (PFGE).

Results: Of the 48,238 *Salmonella* isolates tested from 1997 though 2005, the percent identified as *S.* Newport increased from 0.75% (n = 18) in 1997 to a high of 9.0% (n = 483) in 2003, declining to 3.2% (n = 119) in 2005. The majority (n = 1660; 3.4%) originated from diagnostic submissions followed by slaughter (n = 645; 1.3%) and on-farm (n = 100; 0.2%) sources. Slaughter isolates originated most often from cattle. The percent of isolates resistant to 2 or more antimicrobials averaged 73.5% from 2000 though 2005. PFGE analysis indicated a high degree of heterogeneity among the isolates. Interestingly, isolates which were indistinguishable by PFGE were not necessarily indistinguishable by phenotypic analysis.

Significance: These data indicate that the percent of *S.* Newport originating from animals and submitted to the animal arm of NARMS appears to be declining. However, the MDR phenotype is persisting among a high percentage of the isolates. Continued monitoring and further characterization of isolates is warranted.

T8-02 Barriers to Consumer Safe Food-handling Behaviors

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While recent reports show that food preparation knowledge and some food preparation practices in the home have improved, many consumers still do not follow recommended safe food handling practices. Research has shown that even if a food safety campaign is successful at increasing the knowledge of and attitudes toward safe food-handling practices, actual behavior change often does not follow. The objective of this study was to determine the barriers to safe food-handling practices and improve message design in consumer food safety campaigns. Four focus groups of 45 people, 18–81 years of age were conducted in four urban/suburban and rural communities in New York. Two focus groups were used to gather data on consumer food safety knowledge, attitudes and practices, while the others were questioned about their food preparation behaviors, reasons for non-compliance with recommendations, and reactions to USDA food safety messages. Overall, the focus group participants gave a number of reasons for not following food safety recommendations. Participants relied on longstanding habits, often modeling parents' behavior, and found the additional time and effort required to perform recommended procedures an inconvenience. Perceived risk was low in part because the participants had no personal experiences with foodborne illness. Earlier studies indicate such experience with illness is a significant motivator to comply with recommendations. The USDA "Thermy" campaign received the most positive comments, while respondents were crticial of other USDA Public Service Announcements. Suggestions for increasing behavioral compliance included increasing risk perception, providing information on ways to solve some perceived problems (i.e., how to insert food thermometers into small pieces of meat), and having certain people model appropriate behaviors. Using this information, work is continuing on appropriate message development.

T8-03 Enhancing Food Safety Capabilities in Latin America and the Caribbean through Innovative, On-line, Graduate Level Education

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Effective solutions for today's new and ongoing, multi-faceted and increasingly inter-dependent food safety challenges require unprecedented cooperation between professionals in the fields of law, science, agriculture, health, public administration and education. Design and implementation of a comprehensive and cost-effective approach, in order to enhance food safety capabilities, is vital for the development of Latin America and the Caribbean. Technological and scientific issues of food safety have been the subject of extensive research and training activities. However, continuous outbreaks of foodborne diseases reveal and highlight an unresolved problem. Science, technology and traditional education have not completely satisfied the need for better prevention of foodborne disease in the region. Beyond its ever increasing information-searching power, the internet is another extraordinary tool to enhance food safety education and training. On-line education allows people from different countries, with their correspondingly different realities, cultures, time zones and perspec-

tives, to interact in a virtual classroom, thinking and learning together. The online M.S. degree program on Food Safety Management, designed in collaboration with PAHO and targeting food safety officials in government and industry, will be presented. The Program's curriculum strongly emphasizes managerial abilities though courses such as: Project Management, International Food Law, Marketing Forces and International Commerce, Public Policies Implementation, Management and Leadership, Solution of Controversies, and Information and Communication Technologies. The Food Safety courses focus on advanced HACCP concepts and risk analysis. All managerial courses are given under a food safety approach, along with class examples and research topics selected from current worldwide food safety issues. Some students have experienced improvements in their jobs and networking, leading to better understanding and more effective application of food safety programs in their countries. Such experiences will be described during the presentation.

T8-04 Examining Food Safety Behavior of Food Assistance Recipients Using the Health Belief Model

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Introduction: Poverty and low education levels characterize food assistance recipients, and these factors are assumed to negatively impact food assistance recipients' behavior with respect to food safety. Based on this premise of low food safety awareness and knowledge, as well as poor food-handling practices, educational interventions are prescribed to improve their food-safety behavior. But are food assistance recipients less knowledgeable about food-safety issues and are their behaviors less safe than non-food assistance recipients?

Purpose: To examine food safety risk perceptions, attitudes, knowledge, and behavior of food assistance recipients and compare these to persons who do not receive food assistance.

Methods: We used survey data collected in 2004 and 2005 from North Carolina, Tennessee and Louisiana to generate descriptive statistics, including Chi square test of independence and Anova. Logistic regressions were also used to identify predictors of safe food-handling behaviors. The Health Belief Model served as theoretical basis of modeling food-safety behavior.

Results: Food Stamp and WIC recipients were more likely than persons who received no food assistance to practice safe-food handling. Persons receiving food assistance from food banks and other sources recorded the fewest safe food-handling practices compared to the other groups. The main predictors of safe-handling practices were risk knowledge, risk perception, being a main food preparer, and reading safe-handling labels. Whereas race and food assistance status were not significant predictors of safe washing practices, these factors were important predictors of safe refrigeration and storage practices and, in addition to risk perception, label use and unemployment status.

Significance: The results of this study suggest that food assistance recipients may not necessarily be less knowledgeable than non food assistance recipients, and their food-handling practices may be as good as, if not better than, the practices of persons who do not participate in food assistance program. These results may have implications for the design of education materials for low income food assistance participants.

T8-05 The Effect of Race and Ethnicity on Perceptions of Food Safety among United States Consumers

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Most theories of race and ethnicity in contemporary society suggest that racial and ethnic minorities are more likely than dominant groups to feel concerned about their level of wellbeing. Our study shows that this is borne out in the case of perceptions of food safety. We conducted a telephone survey with 1,013 respondents in the contiguous US at the end of 2005 and the beginning of 2006 using random digit dialing. The data were weighted by gender, age, education, race and ethnicity, and region to obtain a representative sample. Results showed that African-Americans are more likely than other groups to be highly concerned about food safety, and more likely to think about food safety, particularly about the risk of foodborne illness, and to a lesser extent, antibiotic residues, synthetic hormones in meat, pesticides and other chemical residues in foods when they buy food or eat out. Even though white consumers are less likely to be concerned about these issues, both African-American and white respondents were more likely than the other racial-ethnic groups to feel that the current level of foodborne illness in the US is unacceptable. African-American and Hispanic-American respondents are more likely than other racial-ethnic groups to be willing to pay five percent more for their food if the additional cost would lead to a significant reduction in foodborne illness. In summary, African-Americans are most likely to be concerned about the safety of their food and to mistrust the institutions in society responsible for ensuring the safety of their food, with white consumers least likely to be concerned and most likely to be trustful. Hispanic-Americans generally fall between African-Americans and white consumers. These results suggests that further research on actual differences in food safety and foodborne illness between racial and ethnic groups in the US is warranted.

T8-06 Public Perception of Food Handling and Food Safety in Turkey

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Introduction: The responsibility of food consumers in food hygiene has increased significantly in recent decades in Turkey. There is need for valuations of knowledge and attitudes of food consumers in the handling of food.

Purpose: In this study, a survey was prepared to evaluate attitudes of Turkish people living in the most populated area of Turkey in food supplying and preparation in the kitchen. This survey also aimed to obtain the pattern of foodborne diseases surveyors experienced based on their ages, income, sex and education level.

Method: Survey questions were asked of 1,200 people (605 women and 595 men) in 4 different age groups (15–30 years, 31–45 year, 46–60 years, 61–70 years). Questions were generally prepared to expose their knowledge and awareness about food safety and food preparing rules in the kitchen. The survey also evaluated surveyors' opinion about healthfulness and safety of foods sold in supermarkets and about hygiene in food processing plants in Turkey.

Results: According to survey, 45% of those surveyed (mostly 31–45 years old and high income women) are cautious about hygiene and food-handling rules in their kitchens. Only about 32% of study participants have experienced food illnesses, most of it associated with consuming meat and meat products. Most victims were hospitalized. Additionally, 26% of study participants, especially young women, think foods they buy from supermarkets are healthy and safe. Moreover, 15% (mostly 46–60 year olds) believe that food processing plants have fate of hygiene. In this survey, age, sex and education of study participants were found as distinctive factors on most answers.

Significance: In conclusion, in Turkey, most of the high educated and high income level study participants have accurate perceptions and knowledge about food handling and food safety.

T8-07 Costs and Benefits of Implementing HACCP in the Mexican Poultry Processing Sector

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Food safety has become a major issue in international and domestic trades, as awareness of foodborne illnesses has increased among consumers. In addition, outbreaks of animal diseases around the world signal that meat products have to comply with tight food safety controls before reaching final consumers. The HACCP system is an approach to meet this demand for safe food. However, detailed information on costs and benefits of HACCP implementations is needed to provide appropriate advice to food processing plants. The purpose of this study was to evaluate the extent and major costs and benefits of HACCP implementation within Mexican poultry processing plants, and to identify sale destinations of these plants. All thirty-two Federal Inspection Type (TIF) registered enterprises in Mexico were surveyed; 65.6% of them responded. HACCP was totally adopted by 38% of the enterprises, 33.4.% were planning to do it, and the rest were either in the process of implementing it or had no plans to adopt it in the near future. Most of the plants with HACCP indicated that external technical consultants and record keeping were the major costs of implementing and operating HACCP. The main benefit of HACCP programs indicated by the majority of processing plants was reduction in microbial counts. Major supermarket chains took 39.6% of total sales, 22.8% of the sales went to major caterers and only 12.9% of total sales went to international markets (Japan and Central America). Increase and conservation of the domestic rather than the international market has been the driving force to HACCP implementation in the majority of Mexican processing plants. It was concluded that HACCP implementation in the Mexican poultry processing sector was still low, technical assistance was the major cost, and better product quality due to lower microbial counts was the major benefit.

T8-08 Moved to T8-03

POSTER ABSTRACTS

P1-01 Antibacterial Activity of the Lactoperoxidase System against Foodborne Pathogens in Saanen and South African Indigenous Goat Milk

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Introduction: Goat milk is a nutritious food in the tropics; however, its production and handling remains a major problem limiting its consumption. The lactoperoxidase (LP) system has been recommended for preservation of raw milk as an alternative to cooling.

Purpose: This study determined the effect of the LP system on the growth and survival of *E. coli*, *S. aureus*, *L. monocytogenes* and *Br. melitensis* in the milk of Saanen and South African indigenous goats.

Methods: Milk samples were inoculated with either *E. coli*, *S. aureus*, *L. monocytogenes* or *Br. melitensis*. Each milk sample was aseptically divided into two lots; sample A was subjected to activation of the LP system whereas sample B was used as an untreated control. After activation of the LP system, the samples were incubated at 30°C for 6 h and after 6 h, viable bacteria were determined and compared to the initial number of pathogens in the milk samples.

Results: The LP system exhibited bactericidal effect against *L. monocytogenes* and *Br. melitensis* in both Saanen and indigenous goat milk. The LP system showed bactericidal effect against *S. aureus* in Saanen milk; however, it only reduced the rate of growth of *S. aureus* in indigenous goat milk. On the other hand, the LP system only reduced the rate of multiplication of *E. coli* in both Saanen and indigenous goat milk. The same pathogen showed different degrees of sensitivity to the LP system in the milk of the two goat breeds, probably because of the difference in the LP system components in the milk of the two goat breeds.

Significance: The LP system in goat milk could be a promising alternative for controlling the growth of these pathogens, especially during collection of milk at high ambient temperatures and in areas where milk cooling facilities are not available.

P1-02 Control of *E. coli* and Spoilage Microorganisms in Soft Cheese Production Brines Using a Synergistic Antimicrobial Formula

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Introduction: Microbial control of brines used in soft cheese manufacture can help prevent cross contamination of product with spoilage organisms or pathogens. Current antimicrobial options are often expensive or have limited effectiveness.

Purpose: The purpose of this study was to explore new, food-safe antimicrobial additives for use in food production brines.

Methods: One coliform, two yeast, and four mold isolates were cultured from a commercial blue cheese production brine. Brines containing 24% NaCl and various surfactants were inoculated to a level of approximately 10^5 CFU/ml coliform or 10^4 CFU/ml yeast or mold and incubated at 10°C ± 2°C. Populations were determined after four h by plating coliforms to Violet Red Bile Agar and mold and yeast to Dichloran-Rose Bengal-Chloramphenicol Agar.

Results: Spent brine from a commercial blue cheese production line (pH = 4.8) containing 125 ppm (final concentration) of octanoic acid reduced the inoculated coliform, yeast and mold to undetectable levels (< 10 CFU/ml) within 4 h. Spent brine from a commercial mozzarella cheese production line (pH = 5.2) containing 250 ppm (final concentration) of octanoic acid reduced the inoculated yeast, and mold to undetectable levels (<10 CFU/ml) within 4 h, and caused an ~ 2 log reduction in coliform levels.

Significance: Octanoic acid at relatively modest concentrations produced a strong synergistic antimicrobial effect with sodium chloride. This novel, patented formula retains antimicrobial activity in the presence of heavy organic loading in cheese production brines and appears to be effective against yeast and mold, making it a potentially valuable tool for enhancing the shelf life of soft cheese products and possibly as a means to reduce coliform contamination as well.

P1-03 Microbial Evaluation of Commercial Ricotta Cheeses in the City of Campinas

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Introduction: Ricotta cheese, due to its characteristics of high water activity and high availability of nutrients, is a product with a tendency for microbial contamination. Research has shown that *Escherichia coli* is a critical contaminant in this product, especially if it is a pathogenic strain.

Purpose: To verify the presence of total coliforms and *E. coli* in commercial ricotta cheeses in the city of Campinas, São Paulo, Brazil.

Methods: A total of 14 samples of ricotta cheese from different brands were acquired in supermarkets of the city of Campinas, SP. The samples were diluted (10^{-3}) and incubated at 35°C for 24 h in Petrifilm 6404 (3M) for enumerating the total coliforms and *E. coli*. The tests were done in duplicate.

Results: Most of the samples showed high contamination levels for total coliforms and for *E. coli*, with the following results: absence in 14.3% and 35.7% of samples; uncountable in 50% and 7.2% and average contamination equivalent to 4.87 log CFU/g and 4.46 log CFU/g in 35.7% and 57.1% of the samples, respectively. Thus, 64.3% of the samples were above the limit

established by the Brazilian legislation for *E. coli* (Resolution RDC 12/01) that specifies maximum counts of 2.7 log CFU/g.

Significance: The high microbial counts indicate that inadequate pasteurization or contamination after pasteurization by equipment, ingredients or by handlers occurred, and shows the importance of microbial control.

P1-04 *Salmonella* Multi-drug Resistance Isolated from Fresh Cheese

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Introduction: Salmonellosis is one of the most common and widely distributed foodborne diseases. In recent years, the incidence and severity of human salmonellosis has greatly increased, and the emergence of antibiotic resistant salmonellae is threatening to become a more serious public health problem.

Purpose: To determine the spectrum of antimicrobial resistance of *Salmonella* strains isolated from fresh cheeses (panela and adobera) available in Mexico.

Methods: Panela and adobera cheeses, made with unpasteurized milk were obtained from open market vendors in Guadalajara, Jalisco, Mexico. *Salmonella* was detected by the MiniVidas system and isolated using XLD (Xylose-Lysine-Desoxycholate), BG (brilliant green) and SB (Bismuth Sulphite) agars. We examined 140 isolates of *Salmonella* for antimicrobial resistance using the method of Kirby-Bauer. The strains were cultured in tripticase soy broth (TSB). Aliquots (200 µl) were centrifuged and the cells washed with physiological saline solution and inoculated on Mueller-Hinton agar. The plates were incubated at 30°C for 24 h and the haloes of inhibition around different antibiotics were measured.

Results: The incidence of *Salmonella* in cheeses was 30% (16% in adobera, 14% in panela). Out of the 140 isolates tested, 39% were resistant to 5 or more antibiotics, 9% were resistant to fluoroquinolones, 5% were resistant to chloramphenicol, and 9% to ampicillin. Cephalotin and amikacin had the least antimicrobial activity against *Salmonella*. The *Salmonella* serotypes with major antimicrobial resistance were Montevideo, Amsterdam, and Anatum. Fifteen serotypes were identified. Resistance to sulfonamides and trimethoprim was not observed.

Significance: Multi-drug resistant salmonellae are becoming a serious public health concern in Mexico. Resistance to sulfonamides and trimethoprim was not observed in this study and these antibiotics should be limited to clinical use.

P1-05 Katiki – A Traditional Greek Soft Cheese: Survival of *Listeria monocytogenes* and Determination of Biodiversity at Different Temperatures Using Microbiological and Molecular Techniques

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Introduction: *Listeria monocytogenes* is a psychrotrophic Gram-positive bacterium, difficult to control in foods due to its ubiquity in the environment, ability to grow at refrigeration temperature, tolerance to low pH and high sodium chloride levels. It has been associated with several foodborne disease outbreaks due to the consumption of fermented cheese and other dairy products.

Purpose: To determine the fate of acid-adapted and non-adapted *L. monocytogenes* in a traditional Greek soft cheese "katiki" until the expiration date of the product. Changes in the type and levels of the indigenous microbial flora were also assessed either in the presence or absence of the pathogen.

Methods: Samples were inoculated with a five-strain cocktail of *L. monocytogenes* (5.5 log CFU/g) and stored at 5, 10, 15, 20°C over a two-month period (expiration date). Enumeration of the pathogen and microflora was carried out using Palcam, PCA, MRS and M17 agar-based media respectively. Isolates from different storage stages were analysed using Pulsed Field Gel Electrophoresis (PFGE) to determine biodiversity and possible clonal relationships between bacterial strains of the same species in relation to temperature and storage.

Results: Initial levels of lactic acid bacteria which were 6 log CFU/g, slightly increased (1 log CFU/g) and then declined again to the initial concentrations by the end of storage. The concentration of lactococcal populations were similar to initial levels but finally declined by 2 log CFU/g. Survival of *L. monocytogenes* was shown to be temperature dependent, with cells surviving better at lower temperatures. A correlation between the initial autochthonous microflora and the final one was obtained, since phylogenetically related strains were present at both time points (initial and final) examined and at all temperatures. Prevalence of different bacterial strains was dependent on the storage temperature.

Significance: Determination of the pathogen's response in a traditional cheese with wide consumption, and to what extent this response can be influenced by the biodiversity of the product, are important factors in product safety.

P1-06 Incidence of *Listeria monocytogenes* in Milk and Cheese in Macedonia

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Introduction: *Listeria monocytogenes* is a foodborne pathogen of major concern to the milk industry. Macedonian food legislation does not include detection of *L. monocytogenes* in foodstuffs. The incidence of *L. monocytogenes* in milk products in Macedonia was unknown.

Purpose: The purpose of this study was to determine the occurrence of *Listeria* spp. and *L. monocytogenes* in milk and cheese produced in Macedonia.

Methods: The investigation was carried out on 488 different milk products (milk and cheese; cow's and ewe's). Isolation was done according to the Horizontal method for the detection and enumeration of *L. monocytogenes* by ISO 11290-1 2004.

Results: *L. monocytogenes* was isolated from 4.13% of cow's milk, 3.28% ewe's milk, 1.61% cow's cheese

and 1.65% ewe's cheese. In total *L. monocytogenes* was isolated from 2.66% of examined samples. The isolation rate of *Listeria* spp. was 7.99% in all of the examined samples or in 12.4% of cow's milk, 10.66% ewe's milk, 4.84% cow's cheese and 4.13% ewe's cheese.

Significance: These preliminary data suggest that *L. monocytogenes* is present in milk products in Macedonia. Further investigations are necessary to determine the exact prevalence. Expansion of existing legislation is recommended to control the presence of *L. monocytogenes* in milk products in order to protect public health.

P1-07 Microbiological Quality and *Salmonella* and *S. aureus* Frequency in Requesón Expended in Cremerías from Guadalajara City, México

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Introduction: Requesón, a dairy product widely consumed in Mexico, is manufactured by boiling the whey generated from cheese production. The requesón is distributed and commercialized without temperature control in Mexican local markets.

Purpose: To determine the general microbiological quality and the incidence of *Salmonella* and *Staphylococcus aureus* in requesón sold in local markets in Guadalajara, Mexico.

Methods: A total of 84 samples from the main markets of Guadalajara were analyzed. Aerobic plate count (APC), coliforms (OC) and yeast and mold (Y/M) were enumerated using standard plate count agar, violet red bile agar and potato dextrose agar, respectively. Presence of *Salmonella* was investigated with direct enrichment method was conducted by adding 25 g of the sample add 225 mililiter of broth pre-enrichment (broth lactose), respectively transfer 1 mililiter of the mixture to a tube that contains 10 mililiter of tetrationato broth (Bioxon), incubating at 43°C for 24 h, then plating onto brilliant green sulfadiazine agar (Bioxon) and to another one with 10 mililiter of cistina broth selenite, transfer bismuth sulfite agar (Bioxon) and incubating at 35°C for 24 to 48 h and *S. aureus* was conducted by direct plating in Baird Parker Agar.

Results: In 82% of the samples, APC levels were between 7.4 to 8.5 log CFU/g. In 70% of the samples, OC were between 2.5 to 5 log CFU/g. In 74% of the samples Y/M were between 4.3 to 6 log CFU/g. *Salmonella* was detected in 8.4% of the samples. *S. aureus* was present in 88% of the samples at levels between 5.1 to 6.3 log CFU/g.

Significance: The results demonstrate the need to develop and implement food safety controls during processing and distribution of requesón, which should be considered a high risk food.

P1-08 Comparison Study between 3M™ Petrifilm™ Rapid Coliform Count Plates Methods and Desoxycholate Agar Methods for Pasteurized and Processed Milk

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A serious incident of food poisoning caused by contamination of low fat milk occured in Japan in 2000. At that time, several incidents heightened consumers' concerns about the quality of dairy products. To address this situation, the Japan Dairy Industry Association determined they should urgently establish a guideline for voluntary inspection to improve the quality of milk products and to regain trust among the Japanese consumer. The Japanese official method for coliform testing is desoxycholate agar. It must be carefully prepared and requires a 24-h incubation time. In fact, the desoxycholate agar method is not a commonly used agar for food testing in other regions of the world, particularly in countries exporting product to Japan. In this study, 3M™ Petrifilm™ Rapid Coliform Count (RCC) Plates were compared to the Official Japanese Method. For the comparison, *E. coli* IFO 3301, *E. coli* IFO 3972, *Enterobacter aerogenes* IFO 13534, and *Pseudomonas fluorescens* IAM 12001 were inoculated to pasteurized and processed milk at levels of 1.0–9.9/ml and 0.1–0.9/ml respectively, and plated using both methods. Bacterial counts were made following 7, 12, 24, 48 h incubation at 35°C. A Rapid Test Method Assessment Committee was established to review and assess count results and information about practical usage from the study. Results from this evaluation showed no significant difference in coliform counts between the two methods and showed better practical usage with the 3M™ Petrifilm™ RCC plate. The 3M™ Petrifilm™ RCC Plate method provides satisfactory results, in half the time compared to the Official Japanese Method. Acceptance of the 3M™ Petrifilm™ RCC Plate AOAC Official Method 2000.15 by the Japan Dairy Industry Association means that countries exporting dairy products to Japan may feel confident in their use of the 3M™ Petrifilm™ RCC Plate method and results, as these will be accepted in Japan.

P1-09 Evaluation of 3M™ Petrifilm™ Plates for the Detection of Coliforms and *Escherichia coli* in Individual Cow Milk Samples

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Escherichia coli and other coliforms are important pathogens that can cause environmental mastitis in cows. Faster detection of these microorganisms is an important strategy to control damage in mammary glands. The objective of this study was to evaluate the efficacy of the 3M™ Petrifilm™ *E. coli*/Coliform Count Plate to detect *E. coli* and total coliform and 3M™ Petrifilm™ Coliform Count Plate to detect fecal coli-

forms in individual cow milk samples when compared to traditional methodology. The study was carried out in the Experimental Farm located in Minas Gerais State, Brazil. Fifty-five samples were collected from composite quarters of cows after foremilking and teat disinfection using 70% alcohol. Total and fecal coliforms levels were obtained using the Most Probable Numbers (MPN) multiple tubes technique at 30 and 45°C, respectively, compared to results from samples inoculated onto 3M™ Petrifilm™ *E. coli* Plates and 3M™ Petrifilm™ Coliform Plates at 30°C and 45°C, respectively. Results were analyzed statistically using the Chi Square Test. The MPN and 3M™ Petrifilm™ Plates techniques detected total coliforms in 19 and 12; and fecal coliforms in 8 and 6, of the samples tested, respectively. According to the results, the MPN was more efficient ($P < 0.05$) to detect total coliforms; however, both tested methods were considered similar ($P > 0.05$) in the detection of fecal coliforms. Although *E. coli* was not confirmed in the traditional methodology, 3M™ Petrifilm™ *E. coli* Plates showed confirmed results in 24–48 h. MPN methods generally take from 4–7 days to confirm results. These data suggest that 3M™ Petrifilm™ Coliform Plates can be used to detect fecal coliforms quickly. For *E. coli* detection, 3M™ Petrifilm™ *E. coli* Plates may be an important method used to identify infected cows in dairy herds, since confirmed results can be obtained in 24–48 h.

P1-10 DSC Diversity of *Listeria monocytogenes* Ribotypes Isolated from Farmstead Cheese Processing Facilities

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Listeria monocytogenes isolates (152) recovered from environmental sampling of 3 farmstead cheese processing facilities over a 10-week period using three detection/isolation protocols (USDA/FSIS, modified USDA/FSIS (mUSDA), mUSDA with PCR) were characterized by automated EcoRI ribotyping to examine strain diversity within and between plants over time as well as the impact of enrichment media utilized. While most subtypes were consistently isolated by all enrichment procedures, DUP-10144 (of a unique ribogroup) was solely isolated with protocols that utilize *Listeria* Repair Broth in primary enrichment. Eighty-eight isolates, recovered from a single facility, were differentiated into 4 ribotypes (19171, 10144, 19157, and 1042B), in 3 ribogroups. Sixty-nine (78.4%) were identified as DUP-1042B, a known lineage I "epidemic ribotype" which has caused notable outbreaks due to the consumption of pasteurized milk and Mexican style soft cheese among others. DUP-1042B was the predominant isolate from 8/9 positive sites including 2 food contact surfaces (FCS). The presence of this subtype on both FCS and non-FCS suggests cross contamination within the plant. These findings emphasize the potential role of environmental contaminants as sources of end product contamination. While the persistence of specific subtypes in processing facilities has been shown, shifts in population subtypes between samplings in this study demonstrates recontamination of a single site with new subtypes. Furthermore, subtypes of isolates recovered from this plant in 2004 differ from those isolated here. Raw milk was not the likely contamination source as raw milk isolate subtypes from the same farm do not match those from within the processing environments. Analysis of the distribution of ribotypes between plants revealed that each facility had unique contamination subtypes. The use of automated ribotyping can provide useful information on the ecology of different *L. monocytogenes* strains within and between food processing environments that can be used to develop improved control strategies.

P1-11 Detection of *Listeria monocytogenes* in Unpasteurized Liquid Egg Commercially Broken in Japan

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Introduction: *Listeria monocytogenes* is an environmentally ubiquitous foodborne pathogen. It is also well known that the bacterium may contaminate livestock products such as chicken meat. However, there have been few reports about its contamination in eggs or egg products.

Purpose: The purpose of this study was to investigate *L. monocytogenes* contamination in unpasteurized liquid whole egg commercially broken in Japan, and to confirm that the legal liquid egg pasteurization method for *Salmonella* in Japan can be used to control *L. monocytogenes*.

Methods: In 1993, 1994 and 2005, unpasteurized liquid whole egg samples were collected from 13 commercial establishments across Japan. The samples were tested for the presence of *Listeria* species and *L. monocytogenes* by selective enrichment procedures, and the number of *L. monocytogenes* was estimated using the most probable number (MPN) procedure.

Results: Overall, 238 out of 803 samples (30%) were contaminated with *Listeria* spp. The *Listeria* spp. contamination levels varied among the establishments (ranging from 8 to 55% of the samples from each establishment) but did not show differences between 1993–1994 and 2005 (28% and 33%, respectively). *L. monocytogenes* was isolated from 4/803 samples (0.5%), which is considerably lower than known detection rates for other livestock products (5.1~42%). The numbers of *L. monocytogenes* bacteria were further tested for the two contaminated samples collected in 2005, and the contamination levels were both below 7.5 organisms per 25 g.

Significance: This low level contamination leads us to recognize that the legal egg pasteurization method for *Salmonella* in Japan should also be sufficient to ensure inactivation of *L. monocytogenes*, even taking into consideration its higher heat tolerance.

P1-12 *Enterobacteriaceae* and Related Organisms Recovered from Retail Shell Eggs

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Many types of shell eggs are available from retail markets. Some consumers believe that eggs from hens in alternative housing systems (cage free or free roaming) or from organically fed hens will be of superior microbiological quality. Little information is available on the genera of microorganisms that can be recovered from various retail shell eggs. A study was conducted to compare *Enterobacteriaceae* contamination of seven types of retail shell eggs. On three separate days, one dozen of the following egg types were purchased locally and transported back to the laboratory: (A) traditionally fed, housed, and processed, (B) vegetarian fed/nutritionally enhanced, (C) in-shell pasteurized, (D) cage-free DHA enhanced, (E) cage-free, organic, fertile, (F), organic and free roaming, and (G) cage-free, organic, kosher. Eggs (10/type) were aseptically cracked, contents discarded, and the shells macerated with 10 mL phosphate buffered saline. Aliquots (1.0 mL) from individual eggs were duplicate plated using violet red bile glucose agar with overlay and incubated at 37°C overnight to detect *Enterobacteriaceae*. For each presumptive plate, 1–5 colonies were randomly selected and restreaked for purity. Purified isolates were then identified to genus or species using an automated biochemical testing system. There were 205 isolates identified. *Enterobacteriaceae* genera recovered included *Cedecea*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, and *Yokenella*. Closely related organisms identified from retail egg shells included *Aeromonas*, Burkholderia, *Pseudomonas*, Sphingobacterium, and *Vibrio*. Numbers of isolates identified per egg type were: (A) 75, (B) 16, (C) 6, (D) 3, (E) 21, (F) 42, and (G) 42. *Salmonella* were recovered from types A, B, and E. Traditional eggs (A) harbored the greatest number of isolates while in-shell pasteurized (C) and (D) cage-free nutritionally enhanced were the least contaminated. Bacterial contamination of shell eggs appears to be influenced by more than cage system or type of ration.

P1-13 DSC Development of Ozone-based Technology to Eradicate *Salmonella* Enteritidis within Shell Eggs

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Consumption of eggs contaminated with *Salmonella* Enteritidis results in 700,000 cases of salmonellosis and $1.1 billion in losses annually in the United States. Processes available to inactivate *Salmonella* within shell eggs are limited, lengthy, or deleterious to egg quality. Previous experimental evidence, at the bench-top scale level, indicated that shell-egg processing using ozone and mild heat combinations could ensure egg safety and quality. The objective of this study was to develop a pilot-scale ozone-based egg sanitizer and to assess equipment usability to produce *Salmonella*–free shell eggs though sequential application of mild heat and ozone gas. An ozone-processing system was designed, assembled, and set up in a biosafety level-2 pilot plant. The prototype consisted of a high-yield ozone generator, and a stainless-steel vessel (300 liter) equipped with egg-holding trays, air-circulation fan, gas inlet/outlet ports, vacuum pump, and ozone monitor. Shell eggs internally contaminated with *Salmonella* Enteritidis (~10^7 CFU/g egg), using a standardized inoculation protocol, were heated in water (57–59°C) for ≤ 25 min, transferred to the processing vessel, placed under vacuum (10 in Hg), and subsequently treated with ozone gas (10–12% wt/wt O_3 in O_2; ≤ 15 lb/in 2-gauge) for ≤ 40 min. Processing contaminated eggs in the prototype equipment significantly decreased internal *Salmonella* population ($P < 0.05$), when compared to the heat-treated control. For example, heating eggs at 57°C for 25 min followed by vacuum application (10 in Hg), and ozone (~10% wt/wt O_3 in O_2; 10 lb/in 2-gauge) for 40 min, synergistically inactivated *Salmonella* Enteritidis by ≥ 7 log. Selected efficacious combination treatments were found to minimally affect the quality of egg contents. In conclusion, processes relying on sequential application of heat and ozone, in properly designed pilot-scale equipment, can effectively eliminate *Salmonella* in shell eggs. This newly developed system may be considered for future industrial application.

P1-14 Microbiological Safety of Sandwiches from Hospitals and Residential Care Homes for *Listeria monocytogenes* and Other *Listeria* spp.

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Introduction: In the UK, three recent outbreaks of *Listeria monocytogenes* associated with sandwiches purchased from or provided in hospitals have occurred. This is of concern due to the increased risk posed to vulnerable groups, and the severity of disease; hence the focus of this study was on sandwiches served in hospitals and residential/care homes.

Purpose: There is a scarcity of information on the prevalence of *L. monocytogenes* in sandwiches purchased or provided within hospitals and care homes. A study would address this gap in current knowledge, and also highlight to proprietors issues linked to temperature control, growth of *Listeria* spp., and good hygiene practices.

Methods: 3250 sandwich samples collected between April 2005 and March 2006 were examined for the presence and levels of *L. monocytogenes* and other *Listeria* spp. Product and premises information was collected to determine possible risk factors that may be associated with contamination of sandwiches.

Results: The overall contamination rate of *Listeria* spp. in sandwiches from hospitals and care homes was 7.3%. *L. monocytogenes* was detected in 2.7% (89) of samples, 88 at <10 CFU/g and one at 20 CFU/g.

99.9% (3,247) of samples were of satisfactory/acceptable microbiological quality, and 3 (0.1%) were unsatisfactory due to >100 CFU/g of *Listeria* spp. (*L. welshimeri*: 180, 7400 CFU/g; *L. seeligeri*: 1800 CFU/g). Sandwiches from hospitals and care homes were contaminated with *Listeria* spp. and *L. monocytogenes* more frequently when not made on the premises and prepacked, collected from shops and cafeterias within hospitals, or stored or displayed above 8°C.

Significance: This study demonstrates that the control of *L. monocytogenes* in sandwich manufacturing and within storage and handling in hospitals and care homes is critical in order to diminish the potential for this bacterium to be present and multiply in sandwiches to levels hazardous to health.

P1-15 Viability of *Enterobacter sakazakii* in Reconstituted Infant Formula Containing the Lactoperoxidase System

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Neonatal bacteremia and meningitis caused by the opportunistic pathogen *Enterobacter sakazakii* have been associated with the consumption of reconstituted powdered infant formula. A study was done to determine the effects of the lactoperoxidase system (LPOS) and storage temperature on survival and growth of *E. sakazakii* in a milk-based powdered infant formula reconstituted with water. Initially at 0.03 CFU/ml, *E. sakazakii* grew to 2.40–2.74 log CFU/ml in reconstituted infant formula held at 30 or 37°C for 8 h and 0.60 log CFU/ml in formula held at 21°C for 12 h. The pathogen was not detected (less than 1 CFU/227 ml) by enrichment of formula treated with 10–30 μg/ml LPO and stored for 24 h at 37°C or 30 μg/ml LPO and stored for 24 h at 30°C. Populations of *E. sakazakii*, initially at 4.40 log CFU/ml in reconstituted infant formula containing 5 μg/ml LPO, did not change significantly ($P = 0.05$) for up to 12 h at 21 or 30°C. Populations either decreased significantly or were unchanged in formula supplemented with 10 μg/ml LPO followed by storage at 21, 30, and 37°C for up to 24, 8, and 8 h, respectively. Results indicate that LPOS can be used to control the growth of *E. sakazakii* in reconstituted infant formula, thereby potentially reducing the risk of neonatal infections resulting from consumption of formula that may be contaminated with the pathogen. The LPO system, when added to reconstituted powdered infant formula, may also aid in reducing the incidence of other neonatal infections associated with the consumption of infant formula.

P1-16 DSC Inactivation of Human Enteric Viruses and Viral Surrogates in Fresh Salsa Using High Hydrostatic Pressure

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Introduction: Human enteric viruses are a major cause of foodborne illness. Foodborne viruses are commonly associated with raw foods that could be improved with the use of a non-thermal process that inactivates viruses. High hydrostatic pressure (HHP) is a nonthermal treatment that will preserve the raw quality of foods and inactivate pathogens.

Purpose: The objective of this study was to inactivate hepatitis A virus (HAV), Aichi virus (AiV), and feline calicivirus (FCV), a norovirus surrogate, in a fresh salsa using HHP. Ready-to-Eat foods, like fresh salsa, are minimally processed and handled often, making them susceptible to virus contamination.

Methods: Fresh salsa was inoculated with virus then pressure treated. Samples were treated for 1, 5, and 10 min at 9°C at 250, 400, and 500 MPa. Virus was recovered from samples using a vortex method or cationically charged beads and serially diluted. Virus activity was determined using the TCID50 assay in CrFK cells.

Results: No virus activity was observed after a 400 MPa (5 min) treatment for HAV and FCV. HAV was reduced by 7-log and FCV was reduced by 8-log. AiV is more pressure resistant and was not reduced after a 500 MPa (5 min) treatment.

Significance: HHP is an innovative technology that has been shown to inactivate viruses including HAV and FCV, and can be used for Ready-to-Eat foods like salsa which have been implicated in past outbreaks. Parameters must be further investigated to inactivate foodborne viruses like AiV which are more pressure resistant.

P1-17 Comparison and Validation of IS900-sequence and Putative Sequence as a Diagnostic Tool to *Mycobacterium avium* subsp. *paratuberculosis*

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Introduction: Until recently, only a few *M. avium* subsp. *paratuberculosis* (MAP)-specific genes and antigens/epitopes have been identified. The development of a specific immunological test for the detection of MAP is still hampered by the antigenic cross-reactivity between MAP and other mycobacteria species. The specificity of the IS900 transposon as a detection tool has been called into doubt recently due to the discovery of an IS900-like element in non-MAP strains.

Purpose: The objective of this study was to use the real-time PCR to validate and compare a new putative sequence (Mptb52.16) and the IS900-insertion segment as diagnostic tools to identify MAP.

Materials and Methods: Clinical isolates and ATCC reference strains were cultured in Lowenstein-Jensen media. After optimization of the PCR, the DNA extracted from the culture and spiked milk was subjected to real time PCR. Additionally, related mycobacterial strains (*M. fortuitum* subsp. *fortuitum*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. phlei*, and *M. scrofulaceum*) were included to validate the specificity of the target gene(s) as a diagnostic tool.

Results: We were able to amplify both diagnostic targets (IS900 & Mptb52.16) from all MAP strains included in the experiment. Single and Multiplex–PCR of IS900 and putative sequence Mptb52.16 showed the presence of two distinct amplicons of 124 and 156 bp in length, respectively. The new putative sequence was specific only to MAP, whereas the IS900-segment cross reacted with other non-mycobacterial samples.

Significance: The new putative sequence has better specificity than the IS900 sequence as a diagnostic tool to delineate MAP from related Mycobacterial strains. An early, accurate and specific diagnostic protocol will assist the tracking of contaminated herds, food products and would ultimately contribute to reducing exposure.

P1-18 Comparison of a Multiplex PCR with Conventional Method for Monitoring of *Vibrio parahaemolyticus* in Seafoods of South Korea

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Introduction: *Vibrio* strains are the second leading cause of foodborne disease in South Korea, are ever-present in marine and estuarine environments, and are naturally present in or on fish and other seafoods. Typically, human infections with this organism result from the consumption of raw or undercooked seafoods, and Koreans prefer to eat raw seafoods such as sushi, which make *V. parahaemolyticus* infection more common.

Purpose: To compare a multiplex PCR method with a commonly used TCBS method for monitoring contamination levels of *V. parahaemolyticus* in raw seafood.

Methods: We monitored this microorganism in various fisheries products and analyzed a total 208 samples. Detection of *V. parahaemolyticus* in samples by a multiplex PCR amplification procedure targeting the *tox*R gene was conducted and the TCBS method was used in accordance with the Korea Food & Drug Administration's Code of Food Manual.

Results: The occurrence of *V. parahaemolyticus* in seafoods ranged from 0% (March) to 54% (October) and the isolation rates of *V. parahaemolyticus* gradually increased as the temperature rose. The TCBS method, as applied to 50 samples of seafood, resulted in 27 samples that were positive, while 28 samples were positive by multiplex PCR. The multiplex PCR detected other *Vibrios* strains along with *V. parahaemolyticus* in a single assay.

Significance: These data suggest that the multplex PCR, with an elevation in testing and labor time, may provide more rapid and reliable detection of *V. parahaemolyticus* when compared to conventional detection methods.

P1-19 DSC Detection of *Listeria monocytogenes* in Blue Crab Meat (*Callinectus sapidus*) and Blue Crab Processing Plants Using Automated BAX Polymerase Chain Reaction and Standard Culture Method

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Listeria monocytogenes is a foodborne pathogen constituting a major threat to the safety of our food supply. Several methods are used for detecting *L. monocytogenes* in food and environmental samples. However, little information is available about the comparison of BAX polymerase chain reaction (BAX PCR) and Standard Culture Method (SCM) for detection of *L. monocytogenes* in blue crab meat and crab processing plants. The aim of this study was to compare the BAX PCR system with the SCM for detection of *L. monocytogenes* in crab meat and crab processing plants. Samples were collected monthly from seven processing plants during the plant operations (May–November 2006). A total of eight raw crabs, eight finished product and eight environmental sponge samples were collected from each plant during each visit. For detection of *L. monocytogenes* from raw crabs and finished products, enrichment was performed in *Listeria* enrichment broth, whereas for environmental samples, Demifraser broth was used followed by plating on both Oxford agar and *L. monocytogenes* Plating Medium. Enriched samples were also analyzed by BAX PCR. A total of 960 samples were examined; 43 were positive by BAX PCR and SCM. There was no significant difference ($P \leq 0.05$) between the incidence of *L. monocytogenes* in crab meat and crab processing plants detected by both methods. However, SCM performed better for detecting *L. monocytogenes* in raw crabs than the BAX PCR. Six and 7% of environmental samples were positive for *L. monocytogenes* by SCM and BAX PCR, respectively, whereas only 1 and 5 finished products were positive. The results of this study indicate that BAX PCR is as sensitive as SCM for detecting *L. monocytogenes* in crab meat and crab processing plants. While the BAX system provides results in about 3 days, the SCM allows the isolation of *L. monocytogenes* in 6 days.

P1-20 DSC Comparison of the Direct Colony Immunoblot to DNA Hybridization for Enumeration of *Vibrio vulnificus* in Oysters

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Introduction: One of the major pathogens associated with raw oysters is *Vibrio vulnificus* (Vv). The development of a more rapid, reliable and user-friendly method for Vv enumeration is needed.

Purpose: The objective of this study was to compare the rapid direct colony immunoblot (DCI) assay to the DNA probe hybridization method for enumeration of naturally occurring Vv in oysters.

Methods: Oysters (*Crassostrea virginica*) were harvested from the Gulf of Mexico off the coast of Louisiana from April 2006 though November 2006. The oysters were placed at 5°C and Vv counts were determined at 0 h, 4 h, 24 h, 7 and 14 days. Oysters were cleaned and opened aseptically using sterilized oyster knives. Oyster aliquots (150 g–200 g) were mixed with equal amounts of Alkaline Peptone Water. Serial 10-fold dilutions in PBS were made from each oyster homogenate and 100 µl aliquots were plated onto VVA plates. The plates were incubated 16h at 35°C and colonies were counted. *Vibrio vulnificus* was enumerated directly from each VVA plate by direct colony immunoblot (DCI) and DNA probe hybridization (DH) using previously described methods.

Results: There was no significant difference between the DCI and DH methods at 0, 4 and 24 h, with both methods having Vv counts of about 2.90 log CFU/g. By day 7 the counts were (2.62 log CFU/g) compared to the DCI (2.22 log CFU/g). By 14 days the counts for both methods were not significantly different from each other (1 log CFU/g). The DCI method exhibited comparable Vv counts in raw oysters as the DH method except for day 7, which may be due to false positive colonies detected by the DH method.

Significance: The DCI could be a more reliable and user-friendly method for enumeration of Vv in raw oysters.

P1-21 Validation of Post-harvest Processing Using Ultra-low Freezing of Oysters

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Introduction: FDA policy recently mandated post harvest processing (PHP) for oysters harvested from Gulf Coast states in order to reduce the risk of *Vibrio vulnificus* contamination, which is associated with rare but frequently fatal infections following consumption of raw oysters by susceptible persons. PHPs include high hydrostatic pressure, pasteurization, and ultra-low temperature freezing, and validation of PHP requires most probable number (MPN) enumeration of *V. vulnificus* in enrichment broth with species confirmation from isolation on selective medium and DNA probe colony hybridization. There is an urgent need for more efficient and cost-effective assays to facilitate industry compliance with these regulatory guidelines.

Purpose: Ultra-low freezing in liquid nitrogen was examined as a process for reduction of *V. vulnificus* in oysters. Application of quantitative PCR (QPCR) to confirm most probable number (MPN) was compared to standard methods for PHP validation studies.

Methods: DNA was extracted from MPN enrichment broth cultures by a simple boiling method, and QPCR products were detected using SYBR Green I. The assay proved 100% specific and sensitive for *V. vulnificus*, and approximately one bacterium could be detected from overnight growth in enrichment broth. Species-specific confirmation of MPN by QPCR for growth in enrichment broth was comparable (R^2= 0.97) to standard methods and reduced assay time by 2–3 days.

Results: Both assays validated the freezing processes for oysters and showed that the numbers of *V. vulnificus* in oysters were reduced from about 10,000 pre-treatment to 100 MPN/g immediately following PHP, and to ≤ 10 MPN/g by day 21. There was no evidence that QPCR detected residual DNA from dead or dying bacteria.

Significance: This report supports the application of ultra-low freezing for PHP of oysters and the use of QPCR for more rapid analysis of *V. vulnificus* in validation trials.

P1-22 DSC A Novel Technology for Senegalese Fish Preservation by Combined Treatment with Salt and Fermentation Products of Bacteriocin-producing Lactic Acid Bacteria during Storage at 10°C

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The search by developing countries to ensure the safety and quality of their seafood commodities is a great challenge because of the importance of microbiological data to the food trade system. An evaluation of antibacterial activities using combinations of salt with bacteriocins from two recently selected lactic acid bacteria identified as *Lactococcus lactis* subsp. *lactis* (CWBI-B1410), and *Lactobacillus curvatus* (CWBI-B28) was undertaken. Lean and fat Senegalese fish species (sumpat grunt, giant African threadfin, smoothmouth sea catfish) purchased from a local market, were monitored during 22 days of storage at 10°C, by total bacteria counts, using a level of 6 log CFU/g as the end of storage, and those levels compared to those product to which salts (500 + 500 ppm sodium benzoate and sorbate) added. The level of bacteria in fish species was 5.33 log CFU/g. Enteric and H_2S-producing bacteria were dominant during storage of fish at ambient temperatures, whereas lactic acid bacteria became dominant during salt treatment combined with storage at 10°C. The storage of fish in 14% (m/v) saline solution at 10°C resulted in a 1.5 log CFU/g decline of total bacteria counts which were stabilized for 4.5 and 7 days, corresponding to shelf life of fat and lean fish species, respectively. The combinations of salt with sodium benzoate and sorbate, cell-free supernatant of CWBI-B1410 cultures, and cell-free supernatant of CWBI-B28 cultures, delayed the increase of bacteria number, resulting an extension of shelf life of 14, 12 and 8 days for lean fish; 5, 7 and 8 days for moderately fat fish; and 2.5, 7.5 and 13.5 days for fat fish. These data

suggest that this soft technology can be a suitable strategy for enhancing bacterial quality of tropical fish products.

P1-23 Genetic Diversity of *Salmonella enterica* Serovar Weltevreden Isolates from Imported Seafood

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Introduction: Acute gastroenteritis caused by *Salmonella* continues to be a world wide public health concern. A variety of food including poultry, beef, pork, eggs, milk, fish, shellfish, juices, vegetables and fruits have been implicated as vehicles transmitting salmonellosis to humans.

Purpose: The purpose of this study was to assess the extent of genetic diversity among *Salmonella enterica* serovar Weltevreden isolated from imported seafood using antibiotic resistance, genomic restriction fragment analysis by Pulsed-field gel electrophoresis (PFGE) and plasmid profiles.

Methods: Thirty-seven *Salmonella* Weltevreden strains were isolated from imported sea foods from 20 countries during 2000-2005. Isolates were screened for susceptibility to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfisoxazole and tetracycline by the disk diffusion method. Plasmid DNA was isolated by a modified alkaline lysis method and analyzed by agarose gel electrophoresis. For the PFGE, the agarose plugs of total genomic DNA were digested with *Xba*I with or without BSA and fragments were resolved in 1% agarose for 19 h.

Results: Thirty-five of the 37 isolates were sensitive to all the antibiotics tested.

Isolate 611 was resistant to ampicillin and tetracycline, while isolate 631 was resistant to streptomycin, sulfisoxasole and tetracycline. The plasmid analysis of the 37 isolates showed that two isolates (797 and 808) did not carry the plasmid. The remaining 35 isolates were differentiated into 10 distinct profiles based on the presence of different sizes of plasmids. The use of BSA in the restriction enzyme reaction of the PFGE plugs enhanced the activity of the *Xba*I enzyme, allowing complete digestion of DNA in five h. PFGE of *Xba*I restriction digest of DNA from the 37 isolates generated 31 PFGE profiles containing 10 to 15 restriction fragments. Four different PFGE clusters of *Salmonella* Weltevreden were obtained with a genetic similarity ranging from 66% to 76%.

Significance: PFGE patterns obtained were helpful in identifying similar strains isolated from different seafood samples, in different countries and years. The combination of molecular methods (PFGE, plasmid profile) with antibiotic resistance profiles helps to detect genetic changes that can be insufficient to alter the PFGE patterns. Data obtained will be useful in assessing epidemiological spead of infectious diseases and to trace foodborne outbreaks.

P1-24 Eat Puffer and You Will Suffer

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Introduction: Since 2002, a perplexing public health problem had suddenly emerged in the recreational waters of the Indian River Lagoon and Banana River, Brevard County, Florida. It involved Puffer fish previously thought to be safe for human consumption. Professional and recreational fisherman were becoming severely ill and in some instances required hospitization.

Purpose: As a result of the Puffer Fish Poisonings (PFP) the scientific community assembled the resources to conduct intensive research in hopes of determining the source and cause of the poisonings.

Methods: An environmental and epidemiological investigation process was initiated to collect samples of the sealife that inhabited the ecosystem within the Indian River Lagoon and Banana River. Surveillance and extensive inquiries were vigorously pursued utilizing county health department foodborne illness log, Florida Poison Control, local health care providers, hospital infection control practitioners and emergency departments.

Results: The Florida Fish and Wildlife Conservation Commission and United States Food and Drug Administration confirmed *saxitoxin* and *dinoflagellate Pyrodinium bahamense* from the Indian River Lagoon, and in other biota. Toxin profiles revealed *Pyrodinum bahamense*, which was previously not known to be toxic in the United States. Risk communication was critical and imperative and consequently, posters, flyers, press releases, information palm cards and a DVD entitled Puffer fish: Poison Prevention was produced and distributed for public consumption and viewing. Since the release of the locally in house produced DVD no cases of Puffer Fish Posioning have been reported.

P1-25 Bacteriological Quality of Shellfishes Culture Waters Located at the South Bay in Santa Catarina's Island, Brazil

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The incidence of pathogenic microorganisms in seafood such as oysters and mussels, depends mostly on the microbiological quality of the environment in which they are harvested. In Santa Catarina, Brazil, the seafood industry grows annually, highlighting the need for sanitary control of seafood harvesting waters.

The aim this work was to evaluate the bacteriological quality of the culture waters of sea farms located in six different regions of the South bay of Santa Catarina's Island in Brazil. A total of sixty samples were analyzed, from March to December

of 2006, originating from six different culture areas of the region, identified from south to north as A, B, C, D, E, and F. Microbiological analysis for coliforms at 35 and 45°C, using MPN methods described in Standard Methods–21st Edition–2005, was done. From the monthly results, a geometric mean of coliforms at 35 and 45°C was calculated, and the 90th percentile of coliforms at 45°C from the ten collections done in each culture area. The geometric mean of, as well as coliforms at 35 and 45°C were as follows: area A: 2.49 and 2.41 MPN/100 mL; area B: 3.26 and 2.76 MPN/100 mL; area C: 3.13 and 2.52 MPN/100 mL; area D: 15.39 and 5.43 MPN/100 mL; area E: 65.95 and 11.77 MPN/100 mL and at F area: 16.79 and 3.65 MPN/100 mL. The 90th percentile was 5.75 MPN/100mL to the A area; 7.82 MPN/100 mL to B area; 7.35 MPN/100 mL C area; 57.1 MPN/100 mL D area; 163.1 MPN/100 mL E area and 17.6 MPN/100 mL at F area. The coliform averages suggest that the waters in Santa Catarina's South bay are acceptable for shellfish culture and are in accordance with Brazilian legislation, except for Area E, for which the 90th precentile was above the 88 MPN/100ml limit established at the Resolution 357-CONAMA-2005/BRASIL.

P1-26 Depuration in Cold and Ambient Water Changes the Microbiological Profile of Gulf Coast Oysters

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Oysters harvested from the US Gulf of Mexico may contain pathogenic vibrios in warmer months and pose a significant foodborne illness risk for high risk consumers of raw oysters. The objective of this study was to compare the microbiological profile of summer-time harvested raw oysters immediately after harvesting and after two weeks depuration in cold (10°C) and ambient (26°C) salt water (12, 16, and 20 ppt). Oysters were periodically harvested April though September 2006. Three replications were performed for each salinity. Each set of oysters was split, and analyzed either immediately or after a 2-week depuration period. Aerobic plate counts (APC, 32°C, 48 h) were performed using diluted stomached shucked oyster meats in phosphate buffered saline. Dominant colony morphotypes were determined from APC plates (up to 18 isolates per plate). The isolates were identified using gas chromatography of membrane fatty acid methyl esters (MIDI). Salinity level did not impact results; therefore, data for all salinities were combined. At time of harvest, vibrios dominated in fresh oysters (55% of isolates). Depuration in ambient water decreased APC from 5.6 log CFU/g (fresh) to 5 log, while cold water treatment slightly increased counts (5.7 log). Warm water depuration isolates (72 total) consisted mostly of *Vibrio* spp. (31, or 43%) and *Shewanella* (22, or 31%). Warm water depuration produced (31 total) the following pathogenic species: *V. parahaemolyticus* (17 isolates), *V. cholerae* (3) and *V. vulnificus* (1). Cold water depuration isolates (58 total) consisted mostly of *Pseudoalteromonas* (19, or 33%), *Vibrio* (11, or 19%) and *Shewanella* (11, or 19%). Cold depuration vibrios (11 total) were mostly non-pathogenic species, with only 2 isolates identified as *V. parahaemolyticus*. Cold water depuration of raw oysters kept animals alive while reducing the predominance of pathogenic vibrios in the total microflora.

P1-27 Monitoring of *Vibrio parahaemolyticus* Contamination Levels in Seafood, the Southern Area of South Korea, 2006

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Introduction: Among foodborne pathogenic bacterias, *Vibrio parahaemolyticus* has been shown to be the second illness-causing microorganism in South Korea. Foodborne outbreaks by this pathogenic microorganism are usually associated with consumption of contaminated seafood, and Koreans prefer to eat raw seafood such as sushi, which make *V. parahaemolyticus* infection more common.

Purpose: To monitor and quantify contamination levels of *V. parahaemolyticus* in seafood, especially raw seafood.

Methods: We monitored this microorganism in various fisheries products and analyzed a total of 212 samples, which were obtained from Busan (21.7%), Ulsan (20.2%), Suncheon (14.1%), Samcheonpo (27.7%) and Jeju island (16.1%). Isolation of *V. parahaemolyticus* was determined using the Korea Food & Drug Administration's Code of Food Manual and quantitative assay was done using a modification of the Plate Count Method.

Results: The total isolation rates of *V. parahaemolyticus* were 32.08% which was comprised of 32.2% from raw fishes and 31.75% from other seafood. The isolation rates of *V. parahaemolyticus* gradually increase from June (3.2%) and rapidly increased in July (68.96%), as the temperature rose. The Samcheonpo region showed the highest isolation rate (61.8%) and Jeju island (6.25%) was the lowest. Based on quantitative estimates, we can expect an approximate 3.3% rate of *V. parahaemolyticus* contamination of fisheries products. This quantitative data may be used to consider more reasonable microbiological limits and guidelines for *V. parahaemolyticus* in seafood products.

Significance: Quantitative analysis as well as qualitative analysis, like this study, is needed to control foodborne pathogenic bacteria and will need to be revised to allow reasonable distribution and production in the fields.

P1-28 Incidence and Enterotoxiginicity of *Clostridium perfringens* and *Bacillus cereus* from Retail Seafood

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Introduction: *Bacillus cereus* and *Clostridium perfringens* are agents of foodborne illness but little is known about their presence in retail seafood. In the spore state these agents are able to survive certain food processing procedures.

Purpose: The purpose of this work was to determine the levels and enterotoxigenicity of *B. cereus* and *C. perfringens* in retail seafood.

Methods: A total of 311 fresh and processed seafood samples were examined for the presence of these organisms, using FDA Bacteriological Analytical Methods as well as for the genes for non-hemolytic enterotoxin (NHE) components *nheA*, *nheB*, and *nheC* and for the genes for the hemolysin BL (HBL) complex *hblA*, *hblC*, and *hblD* using PCR.

Results: Twelve (4%) of samples contained *C. perfringens* at levels of 3.6 to 240 CFU/g. None of these isolates possessed the enterotoxin gene. These were not further characterized. By comparison 62 of samples were confirmed positive for *B. cereus* at levels of 3.6 to > 1100 CFU/g. Of these, 48 and 27 isolates possessed genes for one of more components of NHE and HBL, respectively. One isolate possessed the gene for the peptide synthetase cereulide (*ces*) for the *B. cereus* emetic toxin, cereulide. 32 (52%) of *B. cereus* isolates were able to produce enterotoxin as determined by the RPLA or ELIZA commercial test kits. Thirteen of the *B. cereus* isolates were able to grow at 12°C.

Significance: The results indicate that, as we have previously shown for retail meat, enterotoxin-positive strains of *C. perfringens* are rare in retail food. However, temperature-abused retail seafood is a possible vehicle for foodborne illness caused by *B. cereus*, especially considering its heat-resistance potential.

P1-29 Rapid Protocol for the Detection of *Salmonella* and *Escherichia coli* O157:H7 in Salad Greens from a Single Eight-hour Enrichment

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Introduction: Because of the implication of produce in recent foodborne outbreaks, reliable methods for the detection of pathogens in vegetables are critical. Successful distribution of short shelf-life foods like spinach and other leafy greens will require well-validated rapid methods for the detection of pathogens.

Purpose: This study evaluated the capability of a shortened enrichment in conjunction with a PCR assay to detect *E. coli* O157:H7 and *Salmonella* from a single enrichment.

Methods: Fourteen samples of freshly picked leafy greens were shipped from a commercial producer to DuPont Qualicon for testing. Each sample type was divided into four 25-g portions. 3 samples were spiked with *Salmonella* and *E. coli* O157:H7 at less than 10 CFU/25 g and 1 was left as the control for each leafy green type. All samples were incubated at 42°C for 24 h. Samples were tested at 8, 10, 12 and 24 h and tested by the BAX® system.

Results: All 14 unspiked samples were negative for both *Salmonella* and *E. coli* O157:H7 at each testing point. For the 42 spiked samples, results at 8 h were comparable to those at 24 h for both *Salmonella* and *E. coli* O157:H7. There was no significant difference between the *E. coli* MP standard and the *E. coli* MP Express protocols.

Significance: This study demonstrates that the BAX® system can detect low levels (< 10 CFU/25 g) of both *Salmonella* and *E. coli* O157:H7 in fresh salad greens after 8-h enrichment.

P1-30 Microbiological Survey of Retail Produce

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Introduction: Recent outbreaks of foodborne illness from consumption of fresh produce have resulted in increased consumer concern regarding the safety of retail produce. There is minimal knowledge regarding microbiological hazards and levels of indicator organisms associated with whole and fresh-cut produce, making it difficult for processors to set microbiological specifications consistent with an industry benchmark.

Purpose: This study was conducted to determine the levels of indicator organisms and prevalence of common pathogens in processed fresh produce samples collected in the Western United States in 2006.

Methods: Packaged green onions, sliced apples, grape tomatoes, mini-peeled carrots, lettuce, spinach, and mixed greens were collected from retail and foodservice locations and analyzed for aerobic plate count (APC), total coliform count (TCC), *Escherichia coli* count (ECC), *E. coli* O157:H7, Enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), *Salmonella* sp., and *Listeria* sp. Petrifilm® plates were utilized to enumerate microbial flora and polymerase chain reaction (PCR) assays were used for pathogen determination.

Results: Mean indicator organism loads ranged from 2.7 to 6.6 log CFU/g for APC, and 0 to 2.1 log CFU/g for TCC. ECC and *E. coli* O157:H7 were not detected in any samples. EHEC was detected at rates of 8.0, 2.3, 12.5, and 0.8% in green onions, apples, carrots, and mixed greens, respectively. STEC was detected at rates of 4.5 4.2, 2.1, and 1.7% in apples, carrots, spinach, and mixed greens, respectively. *Salmonella* sp. was detected at rates of 17.1, 8.3, and 0.8% in tomatoes, carrots, and mixed greens, respectively. *Listeria* sp. was detected at a rate of 1.7% in both lettuce and mixed greens.

Significance: These data present valuable information regarding microbiological hazards associated with specific produce and will assist the industry with establishment of baseline incidence rates and selection of appropriate pathogen testing programs.

P1-31 Effect of Irrigation Water on the Bacteriological Quality of Lettuce during the Growth Period and at Harvest in Norway

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Introduction: In the production of lettuce in Norway, surface water is used as an irrigation water source. Water from such a source is often contami-

nated with fecal bacteria, and therefore is an important source of microbial contamination on lettuce.

Purpose: The aim was to study the effect of irrigation water on the bacteriological quality of lettuce during the growth period and at harvest.

Methods: Three different lettuce producers that use surface water derived from a lake, man-made irrigation pond and a river for irrigation of their respective produce of Iceberg, Frisee and Ruccola lettuce, were the target of this study. Through late May to mid-September, samples of water (71 samples) and lettuce (306 samples) were analyzed for fecal indicators and pathogens (*E. coli* O157, *Salmonella*, *Campylobacter* and *Listeria monocytogenes*) using standard microbiological methods.

Results: Fecal indicators were isolated only sporadically (5% of samples) and in low numbers from the lettuce. *Campylobacter* spp. and *L. monocytogenes* were isolated from one sample each of Frisee. The median water concentrations of *E. coli* in the lake, irrigation pond and river water used for irrigation were 5.1 CFU/100 ml (range <1–1553 CFU/100 ml), 8 CFU/100 ml (range <1–613 CFU/ml) and 127 CFU/100 ml (range 48–>2419 CFU/100 ml), respectively. *Salmonella* Typhimurium was isolated from one lake water sample in the irrigation network. *Campylobacter jejuni* and *coli* was isolated from six (from all three sources), and *L. monocytogenes* was isolated from four of the water samples (from river and pond).

Significance: Although irrigation water may be contaminated, this contamination is not reflected in the bacteriological quality of lettuce.

P1-32 The Proliferation of *Escherichia coli* O157 on Washed and Unwashed Spinach Leaves

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Leafy green vegetables have been implicated in outbreaks of *Escherichia coli* O157-associated diseases. These outbreaks have led to increased scrutiny of vegetable processing and sanitation. Contamination of vegetables with pathogens during processing needs to be further studied because there is evidence of increased proliferation from the time of harvest until transport. The purpose of this study was to determine if spinach contaminated with *E. coli* O157 after leaves have been washed with chlorinated water will contain more pathogen than leaves that have not been washed. Unwashed spinach was purchased from a grocery store, and leaves were sorted into two treatment groups: washed and unwashed. Total plate counts (TPC), *Pseudomonas*, and coliforms counts were taken from three replicates of each treatment, and the replicates from one group were washed with a 100 ppm bleach solution for 30 s and rinsed in neutralizing solution and sterile distilled water. Both unwashed and washed samples were contaminated with a 10^4/ml solution of *E. coli* O157 with a green fluorescent plasmid (gfp) for one minute, and the samples were dried for one hour and TPC, *Pseudomonas*, coliform, and *E. coli* O157- gfp counts were taken. After 24 h at room temperature, counts were taken for each replicate for the treatments, and these were repeated after 48 h. The TPC, *Pseudomonas*, and coliforms from washed and unwashed samples had similar density after 24 and 48 h. The *E. coli* O157 counts on washed leaves were greater than on unwashed leaves ($P = 0.03$) after 48 h but not after 24 h. Sanitation of produce is critical to maintain a safe product, but the cleanliness of the facility is also important to prevent post-wash contamination.

P1-33 Efficacy of Aerosolized Peroxyacetic Acid as a Sanitizer for Raw Spinach

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A serious outbreak of *Escherichia coli* O157:H7 in September 2006 linked to fresh spinach indicated that the safety of fresh produce still needed to be enhanced. Aerosolized peroxyacetic acid as a potential sanitizer for raw spinach was investigated in this study. Spinach inoculated with culture cocktails containing three strains each of *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* was treated with aerosolized peroxyacetic acid (5.42 to 11.42 ≤ gm particle diameter) produced by a commercially available nebulizer for 10, 30, and 60 min in a model aerosol cabinet at room temperature (22 ± 2°C). After exposure, surviving healthy and injured bacterial cells were enumerated on appropriate agars or using the overlay agar method. No significant reduction was found after 10 min exposed to aerosolized peroxyacetic acid ($P > 0.05$). After 30 min of exposure, inoculated spinach experienced a 1.20-log reduction in *E. coli* O157:H7, a 1.01-log reduction in *S.* Typhimurium, and a 0.65-log reduction in *L. monocytogenes* compared to control. After 1 h, log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were 3.50, 3.30, and 3.19, respectively. Similar reduction trends were observed with injured cells. Aerosolized peroxyacetic acid may be a convenient sanitizing method during transportation and storage to minimize microbial contamination, thereby reducing the risk of illness to consumers.

P1-34 Inactivation Kinetics of Inoculated *Escherichia coli* O157:H7 and *Salmonella* Species on Iceberg Lettuce by Chlorine Dioxide Gas (ClO_2)

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Vegetables are an important part of healthy eating. However, vegetables, including lettuce, have been associated with foodborne outbreaks. ClO_2 gas is a promising non-thermal technology for reducing pathogenic and spoilage bacteria on food. The purpose of this investigation was to study: a) inactivation kinetics of inoculated *E. coli* O157:H7 and *Salmonella* spp. on iceberg lettuce by ClO_2 at different concentrations (0.5 – 5.0 $mg l^{-1}$) for 10 min, and, b) the effect of ClO_2 on the quality and shelf life of lettuce during storage at 4°C for 7 days. 100 µl of each targeted organism was spotted onto the surface (5 cm^2) of lettuce (approximately 8–9 log ml^{-1}) separately, air dried, and then treated with ClO_2 gas at 22°C and 90–95% relative humidity for 10 min. Surviving

bacterial populations on lettuce were determined using a membrane transferring method. The inactivation kinetics of *E. coli* O157:H7 and *Salmonella* spp. were determined using first-order kinetics to establish D-values and z-values. The D-values of *E. coli* and *Salmonella* spp. were 2.9 ± 0.1 and 3.8 ± 0.5 min, respectively at 5 mg l^{-1} ClO_2. The z-values of *E. coli* and *Salmonella* spp. were 16.2 ± 2.4 and 21.4 ± 0.5 mg l^{-1}, respectively. A 5-log CFU reduction (recommended by FDA) of *E. coli* and *Salmonella* spp. could be achieved with 5 mg l^{-1} IO_2 gas for 14.5 and 19 min, respectively. Treatment with ClO_2 gas significantly reduced the initial microflora on lettuce and kept them significantly ($P < 0.05$) lower than the control during storage at 4°C for 7 days. However, treatment with ClO_2 significantly ($P < 0.05$) changed the green color of lettuce to white. These results showed that treatment with ClO_2 gas significantly reduced selected pathogens and inherent microorganisms on lettuce; however, the process conditions may need to be altered for consumer acceptance.

P1-35 DSC Non-thermal Pasteurization of Spinach Leaves Using Dense Phase Carbon Dioxide

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While the use of some chemical sanitizers is approved for inactivation of microbes on the surfaces of fruits and vegetables, these compounds often degrade product quality with limited improvement in product safety. Dense phase carbon dioxide (DPCD, high pressure CO_2) is a non-thermal process for inactivation of foodborne pathogens inoculated into various juices and model solutions. Its efficacy has not yet been evaluated for pathogens on produce. DPCD has penetration characteristics that enable inactivation of internalized pathogens that are otherwise protected from conventional chemical sanitization techniques. The purpose of this study was to investigate the ability of DPCD to inactivate *E. coli* K-12, a surrogate for *E. coli* O157:H7, inoculated on fresh spinach leaves. *E. coli* K-12 was inoculated onto baby spinach leaves at a level of ca. 7.2 log CFU/leaf; leaves had approximate surface areas of 9 cm^2. Inoculated leaves were placed in disposable sample cups and then in the pressure vessel of the high pressure CO_2 device. Leaves were exposed for intervals of 0 to 40 min to DPCD (10 MPa, 40°C) at a flow rate of 50 g CO_2/min. After treatment, spinach was blended and serially diluted. Surviving *E. coli* K-12 were enumerated on tryptic soy agar. Significant reductions in *E. coli* K-12 populations were observed with all treatments as compared to controls. The efficacy of DPCD varied significantly with temperature, pressure and treatment time. Inactivation of *E. coli* K-12 to non-detectable levels (> 5-log reduction) was observed for treatments at 40°C and 10 MPa for 20 and 40 min. Discoloration and drying of leaves were observed on samples exposed to DPCD processing for > 20 min. This research demonstrates that DPCD has excellent potential as a pasteurization technology for application to leafy green vegetables, although discoloration and drying issues will need to be addressed.

P1-36 Effect of Antimicrobial Interventions on *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on Leafy Greens

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Introduction: Recent foodborne outbreak events implicating spinach and lettuce have brought about consumer concerns regarding the adequacy of food safety programs for fresh produce. The most common commercial antimicrobial intervention for fresh produce is wash water containing 50 to 200 ppm chlorine as a wash solution.

Purpose: The objective of this study was to compare the effectiveness of chlorine and various other sanitizers for inactivating *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on leafy greens.

Methods: Fresh mixed greens were left uninoculated or inoculated with approximately 6 log CFU/ml of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*. Uninoculated and inoculated mixed greens were treated by immersion for 60 or 90 s in different wash solutions (1:150 w/v) including: chlorinated water (50 ppm) acidified to pH 6.5, acidic electrolyzed water, and acidified sodium chlorite (1,200 ppm, pH 2.5). Following immersion, samples were neutralized, homogenized, and analyzed for survival by standard spread plating on selective media. Each test case (organism x treatment x time) was replicated twice with 5 samples per replicate.

Results: There was no difference ($P > 0.05$) in the effect of time of immersion on the antimicrobial effectiveness of the treatments. Furthermore, there was no difference ($P > 0.05$) in survival of the three organisms regardless of treatment or time. Chlorinated water resulted in reductions in populations of 2.1 to 2.8 log CFU/g on leafy greens. Acidic electrolyzed water resulted in reductions in populations of 2.0 to 2.5 log CFU/g. Acidified sodium chlorite resulted in reductions in populations of 3.0 to 3.8 log CFU/g.

Significance: There has been limited research on the effect of acidified sodium chlorite as a sanitizer for leafy greens and these data indicate it is more effective than conventional application of chlorinated water. These results provide the produce industry with important information to assist in selection of effective antimicrobial strategies.

P1-37 Effects of the Competing Microflora of Fresh-cut Lettuce on Survival and Growth of *Listeria*

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Pathogens, such as *Listeria monocytogenes*, may be present on fresh-cut produce, posing a potential safety problem. In this work, competitive interactions between the indigenous microflora of shredded lettuce and *Listeria* spp. were studied. Co-culture experiments were performed in solid and liquid model media, in which bacterial isolates from the indigenous microflora were tested for possible inhibitory effects. Interactions with individual species

and mixed populations from shredded lettuce affected the survival and growth of *Listeria*. In general, mixed populations diminished *Listeria* growth. Competition between the indigenous microflora and *Listeria* resided mostly with *Enterobacter* spp. and lactic acid bacteria, and not with the pseudomonads. *Enterobacter cloacae* was particularly effective in reducing *Listeria* growth. The effects of gas atmospheres on *L. monocytogenes* and competing microflora were studied using a solid-surface model system. Growth and inhibitory activities of *E. cloacae* and *E. agglomerans* were reduced as CO_2 levels were increased from 0–5% to 10 or 20%. By contrast, the growth and anti-listerial activities of *Leuconostoc citreum* increased under elevated CO_2 concentrations (i.e., 5, 10 and 20% CO_2). It is concluded that complex interactions between elements of the natural background microflora may have significant effects on survival and growth of *Listeria* on produce. These interactions need to be fully understood in order that new mild preservation technologies (e.g., novel gas atmospheres and novel sanitizers/antimicrobials), which may affect the growth or composition of the indigenous microflora, can be safely applied to fresh-cut produce.

P1-38 Effect of Modified Atmosphere Packaging following Treatment of Chemical Sanitizer for Inactivating *Escherichia coli* O157:H7 in Spinach

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Escherichia coli O157:H7-contaminated spinach has recently caused several outbreaks in the USA. However, to date, there has been little research on an effective way for eliminating pathogens in spinach. Therefore, this study was conducted to investigate the effect of chemical sanitizers alone or in combination with modified atmosphere packaging (MAP) on inactivating *E. coli* O157:H7 in spinach during refrigerated storage. Spinach was inoculated with *E. coli* O157:H7 and packaged using four different methods (air, vacuum, CO_2 gas and N_2 gas) following treatment with water, 200 ppm sodium hypochlorite or 200 ppm chlorine dioxide for 5 min at room temperature and stored at 5°C. Then, surviving cells of *E. coli* O157:H7 were enumerated on sorbitol MacConkey (SMAC) agar and on phenol red agar base with 1% sorbitol (SPRAB) for determining chemically-injured cells following treatment and during storage. Treatment with water did not significantly reduce levels of *E. coli* O157:H7. However, treatment with sodium hypochlorite or chlorine dioxide significantly decreased the level of *E. coli* O157:H7, but inhibition was greater when treated with chlorine dioxide compared to sodium hypochlorite. These reduced populations significantly increased in samples packaged in the presence of air. However, reduced populations were maintained when samples were packaged under vacuum, N_2 gas and CO_2 gas during 7 days storage. Packaging under vacuum and CO_2 gas following treatment with chlorine dioxide were most effective at inhibiting levels of *E. coli* O157:H7 in spinach without producing chemically injured cells during storage. These results suggest that the combination of a chemical sanitizer such as chlorine dioxide and MAP may be useful for inhibiting levels of *E. coli* O157:H7 in spinach during storage.

P1-39 Recovery of *Escherichia coli* from Lettuce One Week after Irrigation with Different Types of Water

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Introduction: Irrigation water is often contaminated with fecal bacteria. It has been suggested that contaminated irrigation water is an important source of fecal contamination of lettuce.

Purpose: The aim was to study the recovery of *E. coli* from lettuce irrigated with different types of water one week after irrigation.

Methods: Iceberg and Frisee lettuce, including roots and soil, were collected from one producer, transported to a research field and kept outdoors. The plants were divided in five lots in plastic boxes with drainage holes. Each lot was irrigated once with control water, river water, UV irradiated river water (URW), diluted domestic wastewater (75% ground water; DDW) and UV irradiated diluted domestic wastewater (UDDW), corresponding to 1.8 liter (20 mm) per plant. Fowler's net and plastic for protection for rain was used. Samples of irrigation water were taken directly before irrigation and of lettuce directly before, directly after and 1, 2, 3 and 7 days after irrigation. The samples were analyzed for *E. coli* using standard microbiological methods. The study was repeated once.

Results: The number of *E. coli* in DDW was >2500 CFU/100 ml, while the other water types had numbers of *E. coli* varying between <1 and 685 CFU/100 ml. The number of *E. coli* in Frisee increased directly after irrigation with DDW with subsequent decrease to numbers below the detection limit after 7 days. The same trend was partly seen for Iceberg irrigated with DDW. For the other water types, *E. coli* was detected from two samples each of Iceberg and Frisee irrigated with UDDW.

Significance: The results indicate that irrigation water with high numbers of *E. coli* is needed for recovery on lettuce.

P1-40 DSC Observing *Salmonella* Internalization from Contaminated Seeds and Irrigation Water in Greenhouse Tomatoes

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Introduction: Tomatoes have been implicated in twelve *Salmonella* outbreaks since 1999. Research needs to be done to demonstrate possible points of contamination for the tomatoes during pre and post harvesting.

Purpose: The objectives of this study were to determine the ability of *Salmonella* Montevideo to be internalized in tomato fruit from plants irrigated with *S.* Montevideo solution and to determine whether contaminated seed stock would cause contamination of the fruit. Additionally, the ability of *S.* Montevideo to survive in fertilizer stock solutions was elucidated.

Methods: Tomato plants (5) were grown in pine medium under optimum greenhouse conditions. The study consisted of eight treatment groups: Groups

one through six were watered with 350 ml of a 10^6 *S.* Montevideo solution over 70 days at different time intervals; with group one receiving one *Salmonella* watering at day 0, and group six receiving 6 waterings. Treatment intervals were every 14 days. Group seven was the control and group eight was grown from seeds which had been soaked in a 10^8 *Salmonella* suspension for 24 h, and received no *S.* Montevideo watering treatment. *Salmonella* was also evaluated for ability to survive in concentrated stock and diluted (1.6%) fertilizer solutions.

Results: All tomatoes sampled, regardless of treatment, were negative for *Salmonella* Montevideo using enrichment procedures. Survival of *S.* Montevideo was observed with no significant difference ($P < 0.05$) between solutions and sterile distilled water for all but two fertilizers.

Significance: As shown by this study, *S.* Montevideo is unable to contaminate tomato fruit via irrigation methods or contaminated seed stock. However, it is capable of surviving in fertilizer solutions, stressing the importance of preparing these with potable water. Additional contamination avenues must be examined to elucidate routes to contamination in the field.

P1-41 DSC Effect of Electron Beam Irradiation on Acid-resistant *Salmonella enterica* subsp. *enterica* Serotype Montevideo in Tomato

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Introduction: Increased demand for fresh fruits and vegetables has resulted in changes to production and processing, thus increasing the possibility that these foods may be contaminated with human pathogens such as *Salmonella*. Electron beam (e-beam) is a technology that uses ionizing radiation to destroy bacteria in a non-thermal manner. Therefore, e-beam could be applied to fresh fruit and vegetables to improve microbial safety while simultaneously maintaining the "fresh" characteristics of those products.

Purpose: The purpose of this study was to determine D_{10}-values for acid-resistant *Salmonella* Montevideo subjected to e-beam at four different pHs in homogenized tomatoes.

Methods: *Salmonella* Montevideo was inoculated in homogenized Roma tomatoes. Sample pHs were adjusted to 3.4, 3.9, 4.4, and 4.9. Samples were subjected to e-beam at 0 (control), 0.5, 1.0, 1.5, 2.0, and 2.5 kGy. Survivors were enumerated in duplicate using standard spread-plating method. Survivor curves were plotted on logarithmic scale as a function of e-beam dose for each sample at different pH. The D_{10}-values were calculated as an inverse reciprocal of the slope of the survivor curves. The results were analyzed by analysis of variance and *t*-test.

Results: *Salmonella* Montevideo was below the limit of detection in the samples at pH 3.4 or 3.9. There were significant reduction in survivors ($P < 0.05$) between the control and for samples at both pH 4.4 and 4.9 e-beamed at 1.5, 2.0, and 2.5 kGy. There were significant reduction in survivors ($P < 0.10$) between the control and for all e-beamed samples at both pHs. D_{10}-values for samples at 4.4 and 4.9 were 1.07 and 1.50 KGy, respectively.

Significance: Other research has looked at the effect of e-beam on tomatoes inoculated with non-acid-resistant *Salmonella* Montevideo. The D-values for acid-resistant *Salmonella* Montevideo are considerably higher, indicating that resistance to e-beam increases with acid-resistance.

P1-42 Differences in Physical Characteristics and Survival of *Salmonella* between Round and Roma Tomato Varieties

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At least five large *Salmonella* outbreaks have been traced back to round tomatoes in the US from 1990–2002; however, recent outbreaks have been associated with the consumption of Roma tomatoes. To address why Roma tomatoes have seen an increase in foodborne illness, the present study evaluated the differences in physical characteristics of round vs. Roma tomatoes at different stages of ripeness (green, pink, red). An Instron Model 4411 was used to determine firmness and elasticity, pH was recorded at each stage of ripeness, and the survival of a rifampicin-resistant, five-serovar *Salmonella* cocktail on smooth surface and in puncture-wounds stored for 28 days at 25°C/60% relative humidity (RH) was determined. Results from the Instron testing showed the significant differences ($P < 0.05$) in firmness and elasticity as tomatoes matured in same variety. Roma tomatoes had significantly ($P < 0.05$) higher pH than round tomatoes. The population of *Salmonella* spp. in wounds remained constant with approximately 5.8 log CFU/tomato, while cells on smooth surfaces decreased to undetectable level (2.0 log CFU/tomato) during 28-d storage in same variety. However, this study showed no significant differences ($P < 0.05$) in physical characteristics and survival of *Salmonella* cells between round and Roma tomatoes. Therefore, future researches on the infiltration of *Salmonella* and post-harvesting handling process are needed for better understanding the difference between round and Roma tomatoes.

P1-43 Effects of Heat Treatment on Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on Alfalfa Seeds

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Introduction: Alfalfa sprouts contaminated with *Salmonella* spp. or *E. coli* O157:H7 have been implicated in numerous outbreaks worldwide. High levels of *Salmonella* spp. or *E. coli* O157:H7 on alfalfa sprouts have been traced back to seeds contaminated at low levels from the field. Sprouting conditions of approximately 30°C and high humidity facilitate the rapid growth of both *E. coli* O157:H7 and *Salmonella* spp.

Purpose: The purpose of this study was to investigate the effectiveness of thermal treatments to inactivate *Salmonella* spp. and *E. coli* O157:H7 contained on seeds.

Methods: Alfalfa seeds inoculated with five strains of *Salmonella* spp. or *E. coli* O157:H7 were subjected to dry heat at 55°C for up to 8 days. The treated seeds were sprouted at 30°C for three days and the germination rates were determined.

Results: Greater than five-log reductions in *Salmonella* spp. and *E. coli* O157:H7 on seeds were observed. No pathogens were detected on the sprouted seeds which were initially inoculated with ca. 2 log CFU/g of *Salmonella* spp. or more than 8 log CFU/g of *E. coli* O157:H7. The germination rates of the alfalfa seeds did not decrease after 6 days of heating at 55°C.

Significance: These results showed that heat treatment of alfalfa seeds at 55°C for up to 6 days is effective in enhancing the safety of alfalfa sprouts without significantly affecting germination rates.

P1-44 Comparison of the Efficacy of 1% and 3% Tsunami 100 to 20,000 ppm Calcium Hypochlorite Treatment for Reduction of *Salmonella* on Alfalfa Seed

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Outbreaks of *Salmonella* foodborne illness linked to the consumption of seed sprouts have increased during the past decade. Treatments of the seeds, instead of treating the sprouts, has been shown to be a more practical step in reducing the high numbers of pathogenic bacteria found on the sprouts. The National Advisory Committee on Microbiological Criteria for Food Treatment of seeds using the recommended 10-min exposure to 20,000 ppm $Ca(OCl)_2$ is reported to reduce populations of target bacteria. The objective of the present study was to compare the efficacy of 1% and 3% Tsunami 100 to 20,000 ppm calcium hypochlorite treatment for reduction of *Salmonella* on alfalfa seed. Experiments were conducted using in a commercial seed, a 32-gallon bucket and large mouth media bottles. The 32-gallon bucket method represents a typical method used by sprout producers. The sanitizer solutions were not prepared in buffer to more accurately reflect industry practices. For the commercial methods (commercial seed washer and 32-gallon bucket) of processing regardless of chemical treatment an approximate 1-log reduction in number of *Salmonella* associated with seed was demonstrated. Although the 1-log reduction was significant ($P < 0.05$), differences among the treatments were not significant. Since no buffering solutions were used, the 20,000 ppm $Ca(OCl)_2$ solution had a pH 10–11; most chlorine would be present as OCl^- which is not particularly effective against microbes. Treatment of seed with 1% and 3% Tsunami 100 resulted in similar reductions, 1.77 and 1.34, respectively, in viable cells. The level of reduction between the two treatments was not significant ($P > 0.05$). No viable *Salmonella* were detected in the sanitizer treatment solutions following treatment of seeds or in the wash water following washing of seeds. These results suggest that 1% and 3% Tsunami are as effective as 20,000 ppm of $Ca(OCl)_2$ in the reduction of *Salmonella* associated with alfalfa seed.

P1-45 Evaluation of the Effect of Lactic Acid Bacterial Isolates on the Growth of *Escherichia coli* O157:H7 and *Salmonella enterica* subsp. *enterica* on Alfalfa Sprouts

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Introduction: There have been several outbreaks involving *Salmonella enterica* subsp. *enterica* (SE) and *Escherichia coli* O157:H7 (EC) in sprouts. Conventional treatments may damage sprouts and no acceptable treatments have resulted in satisfactory pathogen reduction. Consequently, competitive inhibition has attracted considerable attention.

Purpose: The purpose of this study was to evaluate the effect of LAB isolates on the growth of SE and EC on alfalfa sprouts.

Methods: Alfalfa seeds were inoculated with pathogen and stored at 4°C. During the seed soak stage of sprouting, the isolates L7 (*Pediococcus parvulus*) and D3 (*Pediococcus acidilactici*) were inoculated (approximately 10^9 CFU/ml) onto the seeds previously inoculated with pathogen. Pathogen levels and pH were evaluated during sprouting and storage.

Results: The only significant inhibition of EC and SE occurred on day 1. The D3 treatment resulted in a 1.50 log reduction in EC and a 1.66 log reduction in SE. The L7 treatment resulted in a 1.09 log reduction in EC and a 1.88 log reduction in SE. However, the inhibition was short lived and was overcome at subsequent sampling times, when there were no differences between treated samples and controls. pH values were significantly different on day 3 for both LAB treatments and both pathogens when compared to the control.

Significance: More investigation is needed to determine if the inhibition observed the first day can be sustained over time. LAB can potentially inhibit both *E. coli* O157:H7 and *Salmonella enterica* subsp. *enterica*. However, during the sprouting process additional controls may be needed after the first day to achieve long-term control.

P1-46 DSC Shelf-life Extension of Strawberries by Use of Ozone-based Combination Treatments

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Strawberries are popular but highly perishable fruits. Fungi are commonly associated with the quality deterioration of strawberries, resulting in significant economic losses. Post-harvest treatments for strawberries are limited, but gaseous sanitizers are potentially useful in improving product shelf life and safety. The objective of this study was to assess the feasibility of using ozone (O_3), carbon dioxide (CO_2), or their combinations, for reducing natural microbiota and extending the shelf life of strawberries.

A gaseous treatment setup, consisting of an ozone generator and a 300-liter chamber equipped with an O_3 monitor and O_3 and CO_2 inlet/outlet ports, was used. Strawberries (~1 lb), in their original plastic container, were treated with O_3, CO_2, or their combination, for 0, 1, 2, and 4 h. In O_3 + CO_2 treatments, CO_2 was fed in the chamber for 5 min at 100 scfh flow rate. The concentration of O_3 was maintained at 10 ppm (vol/vol in CO_2) during each treatment. Control and treated strawberries were evaluated for total aerobic-mesophilic, and yeast-mold counts, as well as overall appearance during storage at 4 and 20°C. The O_3 + CO_2 combination treatments were the most effective in delaying mold growth and quality deterioration of the strawberries. When samples were treated with O_3 + CO_2 for 4 h and stored at 4°C, the initiation of visual mold appearance was delayed until the 16th day of storage, an 8-day extension compared to untreated samples. The differences between combination and control treatments in yeast-mold counts were 1.1 log CFU/g immediately after the treatment and approximately 1.8 log CFU/g after 15 days of storage. The overall difference in total aerobic-mesophilic counts between treated and untreated samples was approximately 1.2 log CFU/g throughout the storage period. In conclusion, treating fresh strawberries with O_3 + CO_2 combination is a feasible technology for extending the shelf life, and potentially improving the safety, of this perishable fruit.

P1-47 Effect of Hot Water Dips on Quality of Highbush Blueberries

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Botrytis cinerea, *Penicillium* spp. and *Colletotrichum* spp. were the dominant fungal pathogens causing decay of "Burlington" blueberry during storage. Hot water dips, effective and non-damaging physical treatments, have been reported for fungal pathogen control in some horticultural products. The objective of this study was to investigate the decay and quality of "Burlington" blueberries (*Vaccinium corymbosum L.*) in response to hot water dips. Blueberries were treated with ambient, 45, 50, or 60°C water for 15 or 30 s along with an untreated control. Fruit were then stored for 0, 1, 2, or 4 weeks at 0°C plus 2 days at room temperature. Microbial quality was evaluated for the aerobic plate count (APC) and yeast and mold count (YMC). Product quality was assessed by measuring the percent of weight loss, split, shriveled, decayed and marketable berries, as well as firmness, pH, soluble solids, titratable acidity and volatile profiles. Treatments slightly affect APC. However, hot water dip at 60°C for 30 s reduced the YMC by 0.2–0.45 log, which represented a 38–64% lower yeast and mold population. Decay incidence was reduced to 2.8, 1.4, 1.2 or 0.6% with ambient water, 45, 50 or 60°C water dips, respectively, compared with 5.1% in controls following 4 weeks at 0°C plus 2 days at room temperature. Weight loss of berries treated with hot water dips was 0.4% versus 3.8% in controls, and number of shriveled berries was 4.9% versus 9.5% in controls ($P < 0.001$). The hot water dip at 60°C for 15 s achieved the most marketable berries, ranging from 94%–95%, followed by 90–96% at 60°C for 30 s and 90–92% at 50°C for 30 s compared with 76%–87% in controls during 4 weeks storage. Hot water dips also altered volatile profiles, and reduced fruit titratable acidity but had no significant effect on fruit firmness, pH or soluble solids content. This study suggests that hot water dips at 50°C for 30 s or 60°C for 15 or 30 s may be used to control decay and maintain blueberry quality.

P1-48 Interventions for Ensuring Food Safety in Mangoes during Phytosanitary Treatments

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Increased consumption combined with increasing risk of foodborne illnesses makes it necessary to identify potential sources of contamination in the food chain and apply intervention processes that prevent/minimize the risk of contamination. The current study investigated the effect of the decontamination treatments with chlorine and lactic acid on the survival of *Salmonella* on the rind and stem scar portions of inoculated mangoes. The presence of the pathogen in the stem tissue (internalization) and the effect of the treatments on the quality (color) of the fruit were also determined. For scar (hydrothermal), a 3.0 log reduction was obtained for control and additional reductions of approximately 2.2 and 1.3 log cycles were obtained with lactic acid and chlorine respectively. Data indicates reduction in pathogen population in cooling for all the treatments except two (Control – increase of 0.3 log, LA-LA – increase of 0.3 log). A 0.5 log reduction was obtained for the control (initial ~4.4 log CFU/10 cm^2) and additional reductions of approximately 1.7 and 1.3 log cycles were obtained for treatments with lactic acid and chlorine respectively during hydrothermal treatment on the rind. For cooling, lactic acid and chlorine gave an overall reduction of approximately 1.3 and 1.4 log respectively compared to control. Although *Salmonella* was not detected in the stem tissue by direct plating method for most of the samples, it was detected after enrichment. Lactic acid was found to be more effective in reducing pathogen population compared to chlorine in all the treatment combinations; however, the sensory aspect (color) of the fruit was compromised.

P1-49 Survival of *Salmonella* Enteritidis in Black Olive Fermentation

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Introduction: *Salmonella* is one of the most common pathogens associated with foodborne illness. Its presence has been reported in a variety of food commodities, but there is hardly any information on the occurence of this microorganism in black olives, a major fermented vegetable in western countries which is primarily consumed without cooking.

Purpose: The purpose of this study was to investigate the ability of *S.* Enteritidis to survice natural black olive fermentation with/without the presence of starter cultures of lactic acid bacteria (*Lactobacillus pentosus*).

Methods: *S.* Enteritidis was added in two different fermentation processes including (i) a spontaneous treatment (i.e., fermentation with the indigenous microflora of olives) and (ii) a treatment with inoculated *L. pentosus* as starter culture. The initial population of the pathogen in the brines was 10^6 CFU/ml. Salt level in the brines was maintained at 6% (w/v, NaCl) while fermentation temperature was kept constant at 20°C. Microbial levels (lactic acid bacteria, yeasts, enterobacteria, *S.* Enteritidis), pH, titratable acidity and organic acids were monitored throughout fermentation.

Results: Inhibition of *S.* Enteritidis was evident in both fermentation procedures. The pathogen population declined rapidly within the first 3 days of fermentation beyond which no cells were detected. In the spontaneous process, a survival period (shoulder) of 1.3 days was evident followed by a sharp decline therafter (estimated death rate 3.7 CFU/day). The presence of *L. pentosus* in the brine resulted initially in lower numbers of the pathogen by approximately 1.5–1.6 log and a lack of survival period although the death rate was lower (2.3 log CFU/day). The presence of salt in the brine and the production of organic acids during fermentation seem to be the main factors that govern the behavior of this pathogen under such stress conditions.

Significance: These data suggest that black olive fermentation either spontaneous or controlled by starter culture is an adverse environment that does not favor the survival of *Salmonella*.

P1-50 Use of Probiotic Starter Culture in Spanish-type Green Olive Fermentation and the Fate of Inoculated *Bacillus cereus*

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Introduction: The use of starter cultures in the Spanish-style green olive fermentation is a practice that becomes more and more familiar to the olive industry with undisputed benefits, while the use of probiotic starter cultures can add extra value to this traditional product. *Bacillus cereus* is a spore-forming pathogen responsible for food poisoning due to production of toxins. Its spores are widely distributed in soil and are therefore potential contaminants of vegetables, including olives.

Purpose: The purpose of the study was to investigate the use of probiotic lactic acid bacteria as starter cultures in Spanish-style green olive fermentation as well as to determine the surviving ability of *Bacillus cereus* vegetative cells and spores in the fermentation environment, with or without the addition of starter culture.

Methods: After completing the debittering procedure, the olives were subjected to a mild thermal treatment and the experimental design for the fermentation included three treatments: (i) spontaneous fermentation, (ii) spontaneous fermentation and inoculation with *B. cereus*, and (iii) fermentation with *L. plantarum* as starter culture and *B. cereus*. The fermentations were monitored for 7 weeks by means of physicochemical properties (pH, titratable acidity, organic acids, sugars) and microbial associations (lactic acid bacteria, yeasts, pseudomonads, enterobacteria, *B. cereus* vegetative cells and endospores).

Results: The use of the probiotic *L. plantarum* proved to be effective for a successive fermentation in terms of final pH and acidity. In all cases, *B. cereus* counts demonstrated an immediate decrease of 2 log-cycles (initial inoculum was 4 log CFU^{-1}) resulting in a 1-2 log CFU ml^{-1} population throughout the experimental period. Similarly, the spore counts were constant during the whole 7-week period (1-2 log CFU ml^{-1}), regardless of treatment.

Significance: The use of a probiotic starter culture appears to be promising and beneficial to the Spanish-style green olive fermentation. On the other hand *B. cereus* seems unable to survive and sporulate in the ecological environment of brine.

P1-51 Evaluation of Chemical Disinfection Treatments for Inactivation of *Salmonella enterica* on Cilantro (*Coriandrum sativum L.*) during Different Storage Conditions

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Produce, including cilantro, has been linked to outbreaks of foodborne illness. There is a need for effective decontamination treatments on fruits and vegetables, as a mean to control microbial pathogens. The objective of this work was to evaluate the efficacy of chemical disinfection to inactivate *Salmonella enterica* on cilantro throughout storage at different conditions of temperature and relative humidity (RH). A 10 g bunch of cilantro was submerged in a cell suspension of rifampicin-resistant *Salmonella enterica* (ca 10^6 CFU/ml) and held at 22°C for 60 min to dry the inoculum. Inoculated cilantro was then stored at 22°C/100 or 60% RH during 3 days, and 4°C/100 or 30% RH for up to 8 days. Periodically, cilantro was treated with water (control), solutions containing chlorine (200 mg/L) or peracetic acid (80 mg/L). At the beginning of storage for all combinations of temperature and H, treatments with chlorine, peracetic acid, and water reduced the pathogen by approximately 2.5, 2.5, and 1 log CFU/g, respectively. There was no reduction in *S. enterica* populations after any treatment on cilantro stored at 22°C (100 and 60% RH) for 3 days. However, after 8 days of storage at 4°C/100% RH, reductions were 2 and 1.7 log CFU/g for chlorine and peracetic acid treatments, whereas at 30% RH reductions were 0.7 and 1.7 log CFU/g, respectively. In general, the inactivation efficacy of the sanitizers decreased as the storage time elapsed. By the end of the storage, the number of cells recovered from cilantro after treatments were not significantly different ($P < 0.5$). The diminished effect of disinfection treatments suggests that microorganisms colonizing cilantro increase over the storage period for the product.

P1-52 Control of *Clostridium botulinum* in Cooked and Packed Rice

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Introduction: More than 88,000 tons/year of cooked and packed rice (CaPR) is produced in Japan. CaPRs are often packaged with oxygen-absorbing agents. We reported that the spores of proteolytic *Clostridium botulinum* were inactivated in rice cooking at 105°C but survived at 100°C (IAFP 2006, Calgary). However, several manufacturers produce CaPRs by cooking at 100°C, and these manufacturers need to control *C. botulinum* by these methods.

Purpose: The purpose of this study is to develop effective methods to control proteolytic *C. botulinum* in CaPR, and to analyze the heat inactivation of the spores in rice cooking.

Methods: Spores of proteolytic *C. botulinum* types A and B were added to the mixture of raw rice and water, then were heated at 100 or 105°C. CaPRs cooked with 25 ppm of nisin or polylysine were inoculated with 10^3/g of spores. CaPRs containing 10^3 spores/g were irradiated with 1, 5 and or 10 kGy of electron beam.

Results: Inactivation greater than 7 log was achieved by 105°C, but a small number of survivors were found after cooking at 100°C and produced the toxins after incubation of the CaPR for 180 days. Thermal inactivation of the spores was faster in rice than in phosphate buffer and cooked meat medium. Though the spores were completely inactivated by irradiation at 10 kGy, rice was brown and had an odor. Neither nisin nor polylysine prevented the toxin production.

Significance: Cooking at 105°C was a significant method to control *C. botulinum* in CaPR, but none of the food additives or the irradiation were effective.

P1-53 DSC Effect of Storage Time and Temperature on the Heat Resistance of *Salmonella* Enteritidis PT 30 on Almonds Exposed to Hot Oil

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Introduction: To date, freshly inoculated almonds have been used to evaluate the survival of *Salmonella* on heated almonds. However, raw almonds may be stored for up to one year before they are processed.

Purpose: The objective of this study was to determine the impact of storage time and temperature on the heat resistance of *Salmonella* on almonds exposed to hot oil.

Methods: Whole Mission variety almonds inoculated with *Salmonella* Enteritidis PT 30 (8.7 log CFU/g) were stored at 4 and 23°C. Inoculated almonds were sampled at 1, 12, 24, 37, and 48 weeks. Almonds (50 g) were 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 (TS) broth, and stomached for 2 min. Appropriate dilutions were plated onto TS and bismuth sulfite agars, and incubated at 37°C for 24 and 48 h, respectively. XLD replica plates were used confirm the population of *Salmonella* on TS agar.

Results: Levels of *Salmonella* on unheated almonds dropped significantly ($P < 0.05$) over 48 weeks by 0.5 and 2.1 log CFU/g when stored at 4°C and 23°C, respectively. Normalized survivor curves were similar over the storage period regardless of temperature but greater variability was observed at 23°C. A biphasic survival curve for *Salmonella* was observed with rapid reduction [3.6 to 3.9 (4°C) and 2.2 to 3.2 log CFU/g (23°C)] in the first 30 s followed by slower but linear decline. The D-values calculated from the linear portion of the curve (0.5 to 2.0 min) were similar at each time point; 0.67 ± 0.09 (4°C) and 0.65 ± 0.13 min (23°C). The time to achieve a 5-log reduction of *Salmonella* at 121°C was calculated to be 1.37 ± 0.18 and 1.97 ± 0.45 min for almonds stored at 4 and 23°C, respectively.

Significance: Freshly inoculated almonds can be used to determine appropriate thermal processes for reducing *Salmonella* on stored almonds.

P1-54 Monitoring Microbial Quality of Organic Watercress in the Production Chain

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Fresh vegetables are important components of a healthy diet, but the number of foodborne disease outbreaks associated with their consumption has increased in recent years. This study was aimed at monitoring the microbial quality of organic watercress at farm level and at industry level (minimal processing - MP). A total of 45 organic watercress samples were analyzed over a period of 12 months for mesophilic and psychrotrophic microorganisms, fecal coliforms, generic *Escherichia coli*, lactic acid bacteria, and *Pseudomonas* spp., as well as presence of the human pathogens *Salmonella* spp., *Listeria monocytogenes* and *E. coli* O157:H7. Fecal coliforms and generic *E. coli* (MPN/mL) were determined in water samples from ponds and wells used for irrigation and washing of vegetables at the farm level and from samples of water in the processing industry. Populations of psychrotrophic microorganisms ranged from 10^5 to 10^7 CFU/g on watercress sampled at the farm and from 10^4 to 10^6 CFU/g for product sampled after minimal processing. Mesophilics and *Pseudomonas* spp. were detected in the range of 10^4 to 10^7 CFU/g, and 10^2 to 10^6 CFU/g, respectively. Lactic acid bacteria showed levels between <10 to 10^5 CFU/g for farm product

and <10 to 10^3 CFU/g after minimal processing. Both washing in the farm and during minimal processing gave 1–2 log reductions of fecal coliforms and *E. coli*. The water used at farm or at processing levels showed low incidence of fecal coliforms and generic *E. coli*. *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7 were not detected in any analyzed sample. Minimal processing does not improve the overall microbiological quality of organic watercress because of the diversity and high population of microorganisms and the structure of this vegetable.

P1-55 DSC Implementing a Dynamic Interdisciplinary Food Safety Curriculum Targeted at Middle School Students

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Engaging young consumers in meaningful learning about food safety is a priority to ensure healthy, lifelong habits at home and in the food service industry. An effective method for delivering food safety education is through instruction in K-12 classrooms. How-ever, students are unlikely to receive meaningful food safety education because teachers lack food safety training and are already over-extended due to high-stakes testing. The purpose of this project was to design an interdisciplinary curriculum targeted at middle schools students that is correlated directly to existing Tennessee and North Carolina state education standards for math, science, language arts, and social studies. The objectives of this project were to: (1) determine how food safety is addressed in public education; (2) identify state standards for core subjects (i.e., math, science, language arts, and social studies) that can be correlated to food safety concepts; and (3) develop, implement, and evaluate an integrated food safety curriculum designed for middle schoolers. A quasi-experimental design (well-matched control group) using pre- and post-participant assessment (e.g., students, teachers) was used to determine changes in content knowledge, behavior, and related dispositions. Teachers were given assessments prior to training, after training, and again after implementation. Student assessments were administered immediately before and after the curriculum implementation and six weeks later. Results of the teacher training workshops show significant changes in knowledge, behavior, and dispositions ($P < 0.05$) with a mean gain of 15.91 points (100 point scale) from pre- to post-assessment. Similarly, students showed significant gains in all four content knowledge areas ($P < 0.001$) with a mean gain of 19 points (100 point scale) from pre- to post-assessment. The outcome of this study is a scientifically validated educational resource that significantly raises food safety awareness and knowledge while providing tangible improvements in food safety behaviors and dispositions.

P1-56 Food Safety Investigation: Applying Food Safety Practices from Farm to Table

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A food safety program for high school students has been developed based on the popular Crime Scene Investigation (CSI) themed television programs. In this two-week supplemental module, which can be incorporated into agriculture, science, and health classes, student investigators solve food safety cases by following clues and applying new knowledge. Using real-life examples and hands-on activities students learn how to apply and implement safe food handling and preparation practices and good agricultural practices (GAPs) while engaged in the module. Initially designed to educate future fruit and vegetable producers, course goals were broadened to include food safety education and fruit and vegetable production and handling procedures that would be beneficial knowledge for all consumers, since approximately only 2 percent of high school agriculture students become farmers. Course content was determined though a Delphi study conducted with experts in food science, horticulture, and microbiology. The course was developed by members of the National GAPs Program in collaboration with the Dpeartment of Education at Cornell University along with nine high school agriculture educators from California, Florida, and New York through a grant from the United States Department of Agriculture, National Integrated Food Safety Initiative. The food safety module was pilot taught in fall, 2006 to students in California, Florida, and New York, and final modifications were made in January, 2007. This presentation will discuss the development of the food safety module, results of the pilot testing, and evaluation mechanisms to determine student knowledge gain. The final curriculum and list of teaching tool kit components will be available at www.gaps.cornell.edu.

P1-57 Consumers' Use and Understanding of Dates on Ready-to-Eat Food Products

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Introduction: There is no universally accepted system for food dating in the United States. However, the US Dept. of Agriculture has posted definitions for "Sell by," "Best if used by," and "Use by."

Purpose: This study measured the frequency with which consumers' read dates on food labels and their understanding of what those dates mean.

Methods: In-person interviews were conducted with 214 shoppers in four states. Using open-ended questions, respondents were asked whether they looked for dates on labels of Ready-to-Eat (RTE) foods, what the phases used on labels mean, and purchase decisions made involving product dates.

Results: Most shoppers reported looking for dates on RTE package labels; however, one third said they were unable to find the date on some foods. Twenty-three percent of the respondents said they bought

a food in the past year after the date on the label, usually because the product was offered at a special price. Many respondents thought the phases "Best if used by," "Sell by," and "Use by" had the same interpretation, and frequently gave the same definition for more than one phase. The most common response for "Best if used by" was "the consumer should not use the food past this date." Most shoppers correctly believed the "Sell by" date was for the store to use; i.e., foods should "not be on the shelf after this date." However, many thought it was still okay to use it within a few days of the "Sell by" date if it was purchased. The majority said that food should not be eaten after the "Use by" date.

Significance: Thus, although consumers had some knowledge of the meaning of product dates, in general they were not always able to distinguish between the different types of dates used.

P1-58 DSC Development and Evaluation of an Educational Bulletin for Consumers Facing Life-threatening Food Allergies

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Food allergies affect 11 million Americans and are responsible for an estimated 30,000 emergency room visits, 2,000 hospitalizations and 100 to 200 deaths per year. Management requires a team effort involving the physician, the patient and the food industry. This study's objective was to analyze recorded interviews with 66 adults with severe food allergies and determine shortcomings in the information provided by physicians and difficulties encountered when eating in restaurants, in order to create an informational brochure for the lay person with a life-threatening allergy. Only 13% of people interviewed had received general information about managing their allergy. Few were familiar with alternative names for common allergic foods. Although self-injectable epinephine is considered vital to prevent death from anaphylactic shock, proper use of self-injectable epinephine device is key to this success. Many respondents were prescribed self-injectable epinephine in case of accidental exposure, but only 63% received training in its use. Many of the interviewees (85%) had experienced a reaction in a restaurant. Although respondents restricted the frequency of dining out, few had a plan to manage their allergy in food service environments. Using information from the interviews, we developed a brief document providing an overview of information that food allergic patients should know with links for more comprehensive information.

The document encourages allergic individuals to be proactive in communicating their conditions and suggests effective strategies. The document was presented to the original interviewees, reviewed, and revised based upon their evaluations. With this packet as a prototype, future plans include designing an educational packet for the food service community focused on how to best serve food allergic patrons. Together, these packets will help prevent accidental contamination by major allergens in restaurants and allow allergy sufferers to be more proactive in describing their condition and managing their health successfully.

P1-59 A Nationwide Study of UK Consumer Attitudes towards Food Safety in the Domestic Kitchen

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Introduction: The domestic kitchen is an important point of origin for the incidence of foodborne disease and food safety education is required to improve food-handling behaviors in the home. A key to the design of effective educational initiatives is an understanding of factors that influence food safety behaviors.

Purpose: Attitudes are important determinants of food safety behaviors and this study aimed to determine consumer attitudes towards food safety in the domestic kitchen.

Methods: Structured in-home interviews of 2,014 consumers were undertaken across the UK, using a computer assisted interview technique. The respondent sample was representative of UK adults aged >16 who prepared meals from raw ingredients at home.

Results: Consumers' homes were perceived as the least likely location for acquiring food poisoning, with 71% unconcerned about food poisoning in the home and 87% confident in their current hygiene practices. Most consumers believed they knew all of the steps for adequate handwashing; drying was perceived to be less essential (46%). A clean kitchen environment was considered important by 96% consumers, although 32% considered that cleaning a chopping board used for preparation of raw poultry under running water would remove all bacteria. Overall, 37% were concerned they might not implement all required food safety behaviors; this varied with age (concern decreased with age), with children (increased concern) and social class (decreased concern with higher social class).

Significance: Cumulatively, attitudes towards food safety were largely positive; attitudinal discrepancies and variations between consumer groups have been identified and highlighted the need for targeted strategies. Findings will be discussed within the context of development of communication initiatives designed to raise awareness of food safety and bring about behavioral improvement.

P1-60 Meals-on-Wheels Food Safety Project

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Introduction: Meals-on-Wheels (MOW) recipients (especially the elderly) may be more at risk for foodborne illness. MOW recipients may save portions of delivered meals longer than recommended and hold them at improper temperatures.

Purpose: The objectives of this project were to conduct a survey of MOW participants for their delivered meal practices, refrigerator food practices and to monitor their refrigerator temperature for one week with a Temperature Data Tracker.

Methods: MOW participants were recruited from 10 counties in Nebraska. After IRB approval and instrument development, an interviewer visited MOW participants, explained the study, placed a Temperature Data Tracker in the participant's refrigerator, and completed the observational form. After one week, the interviewer returned, removed the temperature data tracker, and conducted the survey. Data was analyzed using SAS.

Results: Eighty-one MOW participants completed the study. Sixty percent stated that they usually eat all the food delivered, while 40 percent saved food. Meat and poultry were most often saved for an average of 4 days. Only 4 of the 81 participants had refrigerator thermometers. The average temperature from the Data Trackers was 41.3°F, with an average range of 37.6–49.3°F. Only one reported that the refrigerator did not keep food cold, although 34 refrigerators averaged above 41°F (recommended FDA Food Code temperature); 43 refrigerators averaged above 40°F (recommended home refrigerator temperature).

Significance: This project shows that MOW recipients would benefit from having a refrigerator thermometer to keep food safe in their refrigerators. Education may need to be focused on proper storage of leftovers.

P1-61 Improving the Effectiveness of ServSafe® Courses in Spanish

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In recent years, the need for food safety training for Spanish-speaking restaurant workers has increased significantly. ServSafe® courses in a one-day format have been relatively successful for English speakers. In the last two years the University of Minnesota Extension Service conducted one-day ServSafe® courses in English with exams in Spanish; in 2005, one-day courses in Spanish were offered. However, frequent absences and low passing rates suggest these formats were not effective. In collaboration with the Olmsted County Public Health Dept., McDonalds Corporation, and faculty of the University of Minnesota, the Extension Service developed a new format after identifying barriers to learning: (1) low educational level of students, (2) lack of test-taking skills, (3) little or no time for review of materials before class, (4) lack of collaboration of health agencies and business, and (5) inherent cultural disregard for food safety. Based on these limitations, a ServSafe® Spanish Pilot Plan was developed. The new course consisted of four 2.5–hour sessions given once a week, with three major focus areas: hand-washing, employee illness, and proper cooling of foods. There were hands-on activities, take-home assignments, and practice tests during the first three sessions. The final test was given at the fourth session. At least 90% of the students passed the test and the average passing score was 93%. In contrast, for the one-day Spanish course no more than 40% passed, with an average passing score of 78% (72% needed to pass); for the one-day English course with Spanish test, the numbers were 25% and 78%, respectively. These differences clearly indicate that the four-day ServSafe® format is a more effective teaching format for Spanish-speaking restaurant workers.

P1-62 Comparison of Microbiological Quality and Safety of Products Available to Populations of Different Socioeconomic Status

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Low socioeconomic status has been linked to poorer quality diets and a potential increase in gastrointestinal illness. The objective of this research was to determine if there is a difference in the microbiological quality and/or potential safety of foods available to low socioeconomic status (SES) populations when compared to foods available to high SES populations. A range of products were tested from 3 stores in high SES census tracts and 3 stores in low SES census tracts. Foods tested included Ready-to-Eat (RTE) salads, fresh cut watermelon, broccoli, strawberries, cucumbers, milk, orange juice, fresh ground beef, and raw chicken. The presence or absence of *Salmonella* and *Campylobacter* were determined on raw chicken. All other products were tested for APC, Y&M, coliforms, fecal coliforms, and *Escherichia coli* using FDA's Bacteriological Analytical Manual methods. Overall, with the exception of fresh ground beef, results generally showed higher microbial indicator loads on products from stores in low SES areas. Only a few of these differences, however, were found to be statistically significant. These included: (1) APC ($P = 0.002$) and Y&M ($P = 0.001$) counts in RTE salads, (2) APC ($P = 0.05$) and Y&M ($P = 0.006$) counts in strawberries and (3) Y&M ($P = 0.03$) counts on cucumbers. A difference was also seen in the APC in ground beef ($P = 0.003$), with higher counts seen in high SES stores. Results indicate that there does not appear to be a difference in the level of pathogens that populations of different socioeconomic status are exposed to in their raw food products (i.e., *Salmonella* and *Campylobacter* in chicken and *E. coli* in beef). Our results do indicate, however, that low SES populations may experience produce of poorer microbial quality and may therefore be at greater risk for foodborne illness through these types of products if pathogens are present.

P1-63 DSC Attitudes and Educational Needs Regarding New Alternative Technologies

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Introduction: High pressure, pulsed electric field and ohmic heating are among new alternative methods for processing foods that provided higher nutritional value, longer storage with fresh appearance, and better overall appearance and flavor.

However, there are few such products in the market, the price is slightly higher than traditionally processed food, and there is a limited record of use. Consumer attitudes toward these technologies play a fundamental role for extending the use of these technologies in the food industry. Educational brochures were created about each of these technologies and tested with professional health educators who interact with the public.

Purpose: The purpose of this study was to determine information needs of health professionals and assess satisfaction with a brief technology summary prepared for health professionals.

Methods: A quantitative study with a sample of health professionals was conducted. The study consists on an electronic survey which measured the perceptions of health professionals about these technologies and their evaluation of the educational brochures. Statistical analysis such as ANOVA was used to compare technologies and the acceptance of the educational brochures.

Results: The level of confidence used was alpha = 0.05. Most health professionals were unfamiliar with high pressure, pulse electric field and ohmic heating. Information on product benefits and safety evaluation were of greatest interest to health professionals; however, history and use in other countries as well as worker and environmental safety were also highly valued.

Significance: These findings indicate that health educators today are seeking a wide range of information about food products and food processing. This suggests that educational programs should be prepared to address topics beyond good flavor and nutritional value.

P1-64 What are the Economic Costs of Recordkeeping for Food Processors?

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Introduction: Estimating recordkeeping costs is necessary for determining the cost and paperwork burden of a proposed regulation. Although recordkeeping cost estimates are important for analyzing regulatory impacts, there is no information on current recordkeeping costs in the food industry.

Purpose: The primary objective of this study was the development of a generalizable model to estimate recordkeeping costs incurred by food processors.

Methods: We gathered expert opinion on the likely range of recordkeeping costs from a 14-member expert panel. Our experts' significant experience working at food manufacturing plants allowed them to observe the implications and burden of keeping records. Using a multi-round modified Delphi technique, we elicited labor hour estimates by record type, food sector, and facility size on: writing and updating standard operating procedures (SOPs), initial and refresher training of employees, and record maintenance.

Results: Our expert estimates indicate that overall food processors spend the most time keeping production and process records, followed by QA/QC and laboratory operation records, and incoming raw material inspection records. The total time spent per year maintaining all records ranges from around 800 h for small to 1,900 h for medium to around 4,000 h for large food processors. Processors typically spend between 178 (small) and 425 h (large) writing SOPs upon start-up and between 55 (small) and 141 h (large) updating them on an annual basis. Initial training of employees in recordkeeping procedures ranges from 67 (small) to 291 h (large) on average. Further, processors typically spend between 62 (small) and 260 h (large) on refresher training annually due to employee turnover, new hires, and changes in procedures.

Significance: Our results show that recordkeeping requirements can potentially impose significant costs on food processors. Although these costs increase with facility size, the per-employee burden of recordkeeping is lower for larger facilities.

P1-65 Does Recordkeeping Improve Food Safety?

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Introduction: Records contain information that is valuable for food safety, quality, and other business management purposes. Food processors keep records to comply with FDA and other regulatory requirements, to meet purchaser requirements, improve process control, and conduct trend analyses.

Purpose: The objective of this study was to evaluate the contribution of recordkeeping to food safety in processing plants.

Methods: We used a five-round modified Delphi technique to generate information from a 14-member panel of nationally recognized food safety experts. Each expert had significant experience working in food manufacturing plants, allowing them to assess the implications of keeping records on food safety performance. The panel generated estimates of the relative food safety gains attributable to each record type by plant size. We then used factor analysis to determine the relative importance of record types to food safety.

Results: The relative reduction in food safety risk from a given record type ranges from 0% to 0.7% for small facilities, 0% to 0.8% for medium facilities, and from 0% to 1.2% for large facilities. The highest reductions in risk for large facilities are associated with production and process control records (0.8%), analytic testing records (0.6%), cleaning validation records (0.5%), and equipment cleaning and sanitation records (0.5%). The relative gains in food safety from recordkeeping increase with plant size. Smaller plants typically are less complex, which may result in more efficient oversight of food safety practices. For small plants, the highest reductions in risk are associated with production and process control records (0.5%), cleaning validation records (0.4%), and analytic testing records (0.3%).

Significance: The results suggest that recordkeeping improves food safety in processing plants. Further, the food safety gains from recordkeeping typically increase with plant size, with larger processing plants benefiting relatively more from most types of records.

P1-66 Health Code Violations: Is There Consistency in Types of Violations by State/County, a Case Study

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Introduction: The Food and Drug Administration (FDA) provides recommendations for safe-food handling. Even though the Food Code is not legally binding, it is used by states and local jurisdictions to enact their own regulations. With the diverse range of foodservice establishments, it may be hard for inspectors to apply the same type of subjective analysis to each. Medeiros and Wilcock (2006) state that even objective standards can be influenced by the subjectivity of the health inspector.

Purpose: The purpose of this study was to determine how similar health code violations were, from state to state and county to county, for foodservice establishments.

Methods: Health inspection reports for restaurants from 8 states and 27 counties were used for this study. A coding sheet was developed where health code violations from each of the 3616 inspection reports were entered and could be compared. Ninety different violations were coded. Comparisons were made between states and between counties within each state.

Results: The study found the top five violations for all states were: improperly maintained equipment, floors not clean, handsinks not properly stocked, walls and ceilings not clean, and food not stored six inches off the floor. It is interesting that six of the eight states had 4 out of the five violations as the top five for the state. Nineteen of 27 counties had three of the top five violations as the top five for the county.

Significance: This study showed there could be some consistency between states and counties as to problems found in restaurants. With this consistency, it is suggested that standardizing health inspection reports at the state or national level could be accomplished. This also shows training for inspectors and food service industry personnel is needed in order to concentrate on those critical items.

P1-67 A Model for Assessing the Training Needs of Retail Food Safety Inspection Officers

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In 1998, the US Food and Drug Administration, with input from federal, state and local regulatory officials, industry, trade associations, academia, and consumers, developed the Voluntary National Retail Food Regulatory Program Standards. Since the program standards are based on meaningful public health performance measurements, federal, state, local, and tribal regulatory entities that voluntarily enroll in the program standards use them as a framework for continuous program improvement. Standard 2 of the Program Standards details the essential elements to a training program for retail food safety inspection officers. Based on recommendations from the Conference for Food Protection (CFP), this Standard was recently revised in January 2007 to include a more structured approach (a national best practices model) for field training of regulatory retail food program inspection staff. New hires and/or individuals newly assigned to the regulatory program require consistent training related to the knowledge, skills, and abilities (KSAs) that are applicable to the job being performed. An Assessment of Training Needs (ATN) tool which gauges the retail food safety inspection officer's readiness to conduct independent retail food and foodservice inspections is included in the revised Standard 2. This presentation will provide an overview of the Assessment of Training Needs tool as part of the recommended 5-step training and standardization process outlined in Standard 2 for achieving the required level of inspector competency. By the conclusion of this presentation, participants will be familiar with 5-step training and standardization process outlined in Standard 2 of FDA's Voluntary National Retail Food Regulatory Program Standards.

P1-68 Development of Canadian Standards for Working with Foodborne Viruses: The Microbiological Methods Committee's (MMC) Technical Group on Virology

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Introduction: Known and emerging enteric viral agents such as noroviruses, hepatitis A virus, and hepatitis E are increasingly incriminated in foodborne disease. A pressing need exists to develop more efficient, reliable and rapid methods to capture, concentrate and detect viruses in food matrices.

Purpose: A technical group was created to standardize methodologies for detection and characterization of foodborne viral pathogens and to develop guidelines for validation of newly developed methodologies. To compare disparate methodologies, we propose the use of the feline calicivirus (FCV) as an internal control for all isolation and detection methods targeting RNA viruses.

Methods: Plaque forming units (PFU), tissue culture infectious dose (TCID50) and reverse transcription-polymerase chain reaction (RT-PCR) units were assessed as methods to titre stocks of FCV for use as an internal control. This study was also used as an example of the methodology required for validating viral isolation procedures, including three different viral stocks each tested in triplicate by two different laboratories.

Results: The procedure resulted in successful production of three stocks of similar titres; for any given series of triplicate titrations the titre varied by 3–5 fold. All replicates of the plaque assay gave comparable titres, with maximum differences of 3.5 fold between titres determined by 2 different labs. The TCID50 assay resulted in greater variability, with titres from 1.8×10^6 units/mL to 1.3×10^8 units/mL. Our initial work indicates that plaque assays give a better indication of FCV titre in viral methods. The use of FCV as a universal control for isolation, nucleic acid extraction and downstream amplification methods will be useful in validating current and emerging methods.

Significance: The true potential of the impact of gastroenteric viruses on public health remains uncertain. The use of standardized, continual and active surveillance in Canada will enable quantification and mitigation of this risk.

P1-69 DSC Development of a "Type of Counterfeiters" Hierarchy to Review the Business Case of Anti-counterfeit Food Actions

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Introduction: Counterfeiting is a rapidly increasing US/EU and global problem where an estimated 8% of all global trade is in counterfeit goods. Due to reporting regulations, pharmaceutical counterfeiting is most publicized, but growing incidents are revealing the problem extending to food products. A "hurdle" approach to anti-counterfeit actions is most optimal, including package components, product authentication, traceability or pedigree, supply chain management, manufacturer audits, and incorporating tests in standard operating procedures such as HACCP.

Purpose: The first step in implementing effective anti-conterfeit food measures is to clearly understand the processes and objectives of the offending pathogen, in this case, the counterfeiters themselves. This research expands the single identification of "counterfeiter" into seven genres and to eighteen sub-genres, and develops a tool to analyze proactive anti-counterfeit actions.

Methods: A scholarly review of counterfeit food threats and the counterfeiters was conducted and correlated with expert interviews. The capabilites of the counterfeiters was considered in relation to the effectiveness of RFID, auto-identification, bar coding, and basic mass-serialization.

Results: The research successfully developed a tool to review the effectiveness of specific anti-counterfeit food measures for specific threats by counterfeiter type. The research also successfully developed a "business case" for incorporating anti-counterfeit food analysis in current work processes or auditing policies.

Significance: The US FDA has implemented food traceability requirements under Section 302 of the Bioterrorism Act of 2002, and is attempting to implement traceability for Prescription Pharmaceuticals though the Pharmaceutical Drug Marketing Act. This "business case" and tool will assist, and in some cases is assisting, in optimizing the industry anti-counterfeit actions.

P1-70 DSC Neurotoxic Shellfish Poisoning from Recreationally Harvested Clams in Florida, 2006

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Introduction: Neurotoxic Shellfish Poisoning(NSP) is an illness caused by eating shellfish that have accumulated brevetoxin and its derivatives. The main symptoms include tingling and/or numbness of the lips, tongue, thoat, hands and feet. Symptoms tend to be mild and resolve quickly and completely. It has been confused with a more serious toxin-related illness, Paralytic Shellfish Poisoning. Onset of NSP occurs within a few minutes to a few hours; duration is fairly short, from a few h to several days. Recovery is complete with few sequellae; no fatalities have been reported.

Purpose: This presentation will summarize the etiology of Neurotoxic Shellfish Poisoning, including other shellfish poisoning types, and case study data from an NSP outbreak from recreationally harvested shellfish in Florida in 2006. The cases were apparently unaware of local red tide issues and that harvesting shellfish in the area was illegal.

Methods: During the month of July, 2006, the Lee County Health Dept. received reports of 13 individuals (5 clusters) who experienced neurological symptoms consistent with Neurotoxic Shellfish Poisoning after consuming recreationally-harvested clams from an area not open to legal shellfish harvesting on Sanibel Island and Ft. Myers Beach. The 13 individuals were visitors to the area. Methods of investigation included interviews of cases, descriptive epidemiology, and analysis of clinical samples along with environmental analyses of marine waters.

Results: Leftover clams, clams collected from the area and 2 urine specimens tested positive for brevetoxins. The level of toxicity exceeded the National Shellfish Sanitation Program (NSSP) guidance level of 20 mouse units/100 g.

Significance: Similar NSP incidents have been previously documented in Southwest Florida from recreationally harvested shellfish in 1995, 1996, 2001 and 2005. Tourists and recreationally harvesters are a difficult audience to reach with prevention messages. Besides the media, other means to promote community educational efforts for future red tide events is needed. It might be useful to have public service announcements and other information ready for distribution, emphasizing the fact that cooking these shellfish does not eliminate the toxin. Shellfish should not, under any circumstances, be harvested from unapproved areas. In addition to these efforts, posting signs in all rental units and hotel rooms, warning the public about the risks and legality of self-harvesting shellfish and including contact information for the public to obtain additional information might also be helpful.

P2-01 DSC Changes in Indicator Populations Due to Therapeutic Use of Injectable Antibiotics in Feedlot Cattle

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Introduction: Nationwide, approximately 14.4%, or 4.0 million cattle require the administration of therapeutic concentrations of antibiotics to treat bacterial diseases or bovine respiratory disease (BRD). The effects of therapeutic use of antibiotics

on microbial populations in feedlot cattle are of great concern with regard to public health and food safety. Identification of changes in feedlot cattle indicator microorganisms can provide information on the bacterial load patterns due to therapeutic use of antibiotics.

Purpose: The purpose of this study was to determine which treatment(s) affect the bacterial load patterns of indicator populations (i.e., *Escherichia coli, Enterococcus, and Salmonella*) of feedlot cattle after the administration of therapeutic injectable antibiotics.

Methods: Five commonly used injectable antibiotics (Excede, Nuflor, Biomycin, Baytril, and Micotil) were administered therapeutically to feedlot cattle. Fecal samples were collected from animals prior to treatment for naturally-occurring BRD and 96 hours after treatment on fifty animals per exposure (treatment) and fifty (control) clinically healthy pen mates of the treated animals. A direct-plate count method was used to enumerate enterococci and *E. coli* whereas a most probable number (MPN) technique was used to enumerate *Salmonella*. Descriptive statistics were generated for bacterial counts by treatment.

Results: There were statistically significant differences ($P < 0.05$) between the control and five treatment groups. Reduction of populations of *E. coli* occurred between 1-3 log cycles and reduction of *Enterococcus* at 1-2 log cycles were observed in cattle treated with all the antibiotics. The greatest reduction in *Salmonella* populations, when *Salmonella* was present, was observed with cattle treated with Excede, Nuflor, and Baytril.

Significance: These results provide important information on the effects of commonly used injectable antibiotics routinely given to feedlot cattle and the effects on specific indicator populations. These results also provide knowledge that therapeutic antibiotic use does reduce indicator populations commonly associated with antibiotic resistant bacterial strains.

P2-02 DSC Pre-harvest Control Factors Affecting Prevalence of Shiga Toxin-producing *Escherichia coli* in Cattle Grazing Irrigated Pastures

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Worldwide, contamination of foods with Shiga toxin-producing *Escherichia coli* (STEC) caused numerous outbreaks of human illnesses. The illnesses ranged from mild or bloody diarrhea to the life-threatening hemolytic uremic syndrome (HUS). Because cattle are STEC reservoirs, their fecal shedding of these pathogens will continue to be a food safety concern, as shown in the recent beef and produce outbreaks. To combat the problem, it is essential to identify and implement on-farm factors that decrease cattle shedding of STEC. This study was designed to determine effects of pre-harvest factors on STEC prevalence in California cattle grazing irrigated pastures. In four cow-calf operations (ranging from 38 to 1,300 cows), 638 fecal samples were collected from 437 cows and 201 calves (28 to 92 days old) over one year. Approximately 40 samples were collected from each operation in each season. Prevalence rates of STEC were higher ($P < 0.05$) for calves than for cows (7.5 vs. 1.6%) and also higher ($P < 0.05$) in the spring than in the remaining seasons (7.4 vs. an average of 2.3%). Overall, the STEC isolates belonged to nine serotypes (O1:H2, O26:HUT [untypeable H antigen], O125:H2, O125:H19, O125:HUT, O169:H19, OUT [untypeable O antigen]:H16, OUT:H^- [nonmotile isolate], and OUT:HUT). Of these serotypes, two (O26:HUT and OUT:H^-) caused HUS, three (O1:H2, OUT:H16, OUT:HUT) caused other human illnesses, and five (O1:H2, O125:H2, O125:H19, O125:HUT, and O169:H19) have not been reported previously in cattle. *E. coli* O157 isolates were not found in the cattle tested. Of the various management practices tested, lower ($P < 0.05$) risks of STEC shedding were associated with offering running drinking water (streams or springs vs. ponds or ditches) to cattle and with shortening the calving season to ≤ 2 months. Improving management of grazing cattle appears to decrease the risk of carriage and fecal shedding of STEC.

P2-03 *Salmonella* Contamination of Cattle between Feedlot and Abattoir

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Introduction: Salmonella enterica is an important foodborne pathogen that has been associated with the consumption of beef. *Salmonella* can be found in healthy cattle at slaughter and may contaminate carcases during processing.

Purpose: The presence of *Salmonella* on cattle, transport vehicles and abattoir holding pens was investigated to determine potential sources of cattle contamination during transport and lairage.

Methods: The incidence and number of *Salmonella* in the feces and on the hides of cattle was determined for 3 groups of 30 animals, both at a feedlot and at an abattoir immediately after slaughter. Samples from the carcases of the cattle prior to chilling were also collected. The presence of *Salmonella* on the sides and floors of the transport vehicles and abattoir holding pens was determined prior to entry of the cattle.

Results: Salmonella was not isolated from the hides of cattle sampled at the feedlot or the hides or carcases sampled at the abattoir. *Salmonella* was isolated from 2% of 90 cattle feces collected from the abattoir but not from feces collected at the feedlot. *Salmonella* was isolated from the floors (4%) and sides (11%) of trucks and from the floors (33%) and rails (26%) of the abattoir holding pens. Counts of *Salmonella* determined using an MPN technique were low with detectable numbers occurring only in samples collected from the abattoir pen floors (maximum count of log -2.03 MPN/cm^2).

Significance: The isolation rate of *Salmonella* from animal hides and feces did not increase significantly between the feedlot and after slaughter in this study. The presence of *Salmonella* in the transport and lairage environments suggests these are potential sources for cross contamination of animals although no direct evidence of this was found in this study.

P2-04 Survival and Growth of Different Strains of *Escherichia coli* O157:H7 in Cattle Water Troughs

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Escherichia coli O157:H7 is an emerging foodborne pathogen, with cattle being implicated as the major reservoir for this organism. *E. coli* O157:H7 is a very persistent pathogen and has been shown to survive for long periods of time in the cattle farm environments. The purpose of this study was to investigate the survival of different strains of *E. coli* O157:H7 in cattle water troughs. Experimental microcosms were set up to evaluate survival of three environmental and two human strains of *E. coli* O157:H7 in the sediments of cattle water troughs in the presence and absence of natural microflora at 5, 15, 25, and 37°C. An inoculum of each of the five strains of *E. coli* O157:H7 was added to each separate aliquot of the sediment sample and mixed to obtain a bacterial concentration of ~10^7 CFU/g. At all temperatures, duplicate samples were incubated and the 1 g aliquots of samples were cultured to determine the presence of *E. coli* O157:H7 periodically for 60 days. Higher temperatures (25°C and 37°C) resulted in significantly greater ($P \leq 0.05$) decrease of *E. coli* O157:H7 than lower temperatures (5°C and 15°C), especially in the presence of natural microflora. After 60 days, all the strains were detectable by direct culture method in the presence of natural microflora at 5°C and 15°C, but could not be recovered when incubated at 25°C and 37°C. It was also observed that the growth and survival of human *E. coli* O157:H7 isolates was significantly lower ($P \leq 0.05$) than environmental isolates at lower temperatures (5°C and 15°C) both in the presence and absence of competing microorganisms. This study shows that *E. coli* O157:H7 can survive for long periods of time in cattle water trough sediments even at low temperatures and emphasizes the importance of improving sanitary conditions of the water troughs.

P2-05 Feedlot Dust as a Source of Cross Contamination of *Escherichia coli* O157 on Beef Feedlot Cattle Hides Prior to Shipping

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Introduction: Farm animals are considered the primary reservoir of *E. coli* O157. Hides are the primary source of contamination of the carcass, so an increase in pathogen loads on the hides can result in an increased amount of contamination of the final product

Purpose: Cattle pass though a significant amount of dust during loading. The objective of this study was to determine if this could potentially be a source of cross contamination of the animals during loading and if control of this dust could control the amount of pathogen contamination.

Methods: 250 cattle were sampled over a 4-day period. Animals were loaded though a loadout area where dust was generated or though a clean, concrete loadout area where no dust was generated from animal movement. We collected composite hide samples from the withers and midline. Air samples were collected as well as soil samples in the snake area and from the pens. We determined the presence and the quantitative amount of *E. coli* O157 in the samples.

Results: In the clean loadout area, 15% of the air samples tested was positive was for *E. coli* O157 while 57% of the samples collected in the dirty load-out area tested positive. The prevalence of *E. coli* O157 was 45.7%, 58.3% and 80.5% in home pens, loadout area 1 and loadout area 2, respectively. The total *E. coli* O157 numbers on the hides increased after exposure to the dust cloud. In the dirty loadout areas the total *E. coli* O157 loads on the hides increased by 1.2 log cycles from 2.5 log CFU/cm^2 to 3.7 log/cm^2.

Significance: The dust in the loadout area is a source of contamination of the hides and control of this dust could reduce the amount of pathogens that are carried on the hide to the slaughter facility.

P2-06 Fate of Zoonotic Pathogens in Static Composting Piles of Chicken Litter and Peanut Hulls

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Introduction: Inadequately composted manure has been cited as a potential pre-harvest source of contamination of produce. Operations that do not turn, or that provide only limited turning of, the compost material are likely to have increased survival of zoonotic pathogens.

Purpose: The fate of avirulent pathogens (gfp-labeled *Escherichia coli* O157:H7 and *Listeria innocua*, and rifampin-resistant *Salmonella* Typhimurium) in the field was monitored at both interior and surface sites of static composting piles composed of chicken litter and peanut hulls.

Methods: Six static compost piles, having a base diameter of 3 – 4 m each, were formed, using chicken litter and aged peanut hulls, and mixed to give initial C:N ratios ranging from 15 – 18. Subsamples of the initial mixture were removed, sprayed with one of the pathogen inocula to give concentrations of 10^5 – 10^7 CFU/g, distributed into gas- and water-permeable packages, and then positioned either in the interior (30 cm from both the surface and ground) or on the surface (30 cm from the ground). Temperature and oxygen readings were taken on compost piles while packaged compost material was monitored for pathogens, pH, and moisture.

Results: In interior samples, pathogens were inactivated quickly, being detectable after 2 days of composting by enrichment culture only. Despite exposures to elevated temperatures, however, *Salmonella* continued to be detected by enrichment up to 14 days after composting was initiated in interior

samples. In surface samples, the fate of pathogens was dependent on the season and ambient temperature conditions in which composting was conducted. During the summer, pathogens were detected after 4 days of composting by enrichment culture only whereas > 2 log CFU/g remained viable for up to 28 days in surface samples of piles composting during the fall.

Significance: Significant survival of pathogens occurs on the surface of unturned static compost piles containing chicken litter such that the material should be held for more than one month before applying to fields.

P2-07 DSC Inactivation of *Salmonella* and *Listeria* in Dairy Manure-based Compost

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Introduction: Foodborne pathogens such as *Salmonella* spp. and *Listeria monocytogenes* are often present in animal manure. These bacteria can contaminate fresh produce though the common practice of using raw or inadequately composted manure as soil amendments for vegetable production.

Purpose: The objective of this study was to determine the fate of *Salmonella* sp. and *Listeria innocua* in dairy manure-based static compost heaps in a field setting.

Methods: Avirulent *Salmonella* Typhimurium 8234 and *Listeria innocua* 33090 strains selected for rifampin resistance were inoculated into dairy manure-based compost mixtures. The inoculated compost mixture was individually packaged and placed within compost heaps at top, middle, and bottom locations. Surface samples were secured to the compost heaps in individual sampling trays. Bacteria were enumerated by direct plating on XLT-4 and Oxford media containing rifampin (100 μg/ml), for *Salmonella* and *Listeria*, respectively, or detected by selective enrichment cultures.

Results: *Salmonella*, at initial cell numbers of ca. 5 log CFU/g, was undetectable after 1, 1, and 3 days of composting at the top, middle, and bottom locations, respectively, of compost heaps. For the high inoculation trial (ca. 7 log CFU/g), both *Salmonella* and *Listeria* were undetectable at all three internal sampling locations after 2 days of composting. In each trial, however, both salmonellae and listeriae in surface samples were detected though 4 months. Additionally, temperatures for all internal heap locations were greater than 50°C for 12 to 13 days during the first two weeks of both trials.

Significance: Results reveal that both *Salmonella* and *L. innocua* at initial cell numbers of 5 to 7 log CFU/g can be inactivated within 3 days in dairy manure-based compost heaps that were greater than 50°C during this period of time. Importantly, the pathogens can survive for at least 4 months on the surface of unturned static compost heaps.

P2-08 Virulence Genes and Enterohemolysin Production by *Escherichia coli* Isolates Derived from Feedlot Beef Cattle, Environment and Carcasses

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Several outbreaks have been caused by serotype *E. coli* O157:H7; however, non-O157 strains have also been incriminated. The inability of O157 to ferment sorbitol is one of the characteristics used to differentiate this serotype from others. The objective of this study was to evaluate the presence of some virulence genes in, and the capacity of hemolysin production by, sorbitol positive (s+) and negative (s-) *E. coli* isolates. A total of 615 isolates (332 s+; 283 s-) obtained from feedlot cattle, from the growing environment and from their carcasses were examined. The presence of *stx*1, *stx*2, *uid*A and *eae*A were evaluated by multiplex PCR, and *ehx*A was evaluated alone. The ability to produce hemolysin was done by stabbing on blood agar. Virulence genes and/or enterohemolysin were detected in 86 isolates (13.9%) (51 s+). These isolates were from feces, water, carcasses and the floor of the cold room where the carcasses were kept. 21 isolates (10 s+) produced hemolysin but presented none of the virulence genes and 25 (22 s+) showed *stx*1. Gene eaeA was detected in 20 isolates (4 s+), and *stx*2 in 17 (5 s+). 13 isolates (1 s+) presented *ehx*A and were hemolysin producers, while 8 s+ isolates harbored *ehx*A but did not express it. Among the 5 isolates presenting *stx*1 and *stx*2 simultaneously 4 were s+. *uid*A, a gene specific for O157:H7, was found in 7 s- isolates all from animal feces. However, one of these was serotyped as O97:H1. Serotypes identified were: O97:H1 (6 isolates); O38:H21 (4); O85:H7 (4), O136:H16; O172:H-; ONT:H16; ONT:H34; ONT:H7; ONT:H2; ONT:H8; ONT:H31; ONT:H34; ONT:H21; ONT:H10; ONT:H46; O178:H-; O157:H12; O85:H7; O178:H7, O26:H-; O79:H14; O177:H-; O102:H21; O87:H25. The results indicate, for the first time in Brazil, the presence of O157:H7 in the feces of feedlot beef cattle.

P2-09 Dynamics of *Campylobacter* Spread Investigated in Fourteen Broiler Flocks in Switzerland

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Introduction: Poultry is assumed to be among the most important sources of foodborne *Campylobacter* infections. Broilers are often carriers of *C. jejuni* and may contain large amounts in their intestines when colonized. Horizontal transmission is generally

believed to be the common way for colonization, but the epidemiology of *Campylobacter* in broiler production is still not completely understood.

Purpose: The aim of this study was to investigate the spread and distribution pattern of *Campylobacter* within different broiler flocks during the rearing period.

Materials: Ten conventional and four extensive outdoor broiler flocks, distributed over nine farms, were investigated twice a week during a 35–58 day rearing period. Isolation of *Campylobacter* was accomplished by culture after enrichment (*Campylobacter* Selective Broth and Agar). *C. jejuni* was identified by its positive reaction in hippurate hydrolysis. Isolated strains were (i) genotyped by restriction fragment length polymorphism (RFLP) analysis of the *flaA* gene using *Dde*I and macrorestriction profiling with pulsed field gel electrophoresis (PFGE) using *Sma*I.

Results: Of the 4,112 collected samples, 157 (3.8%) were *Campylobacter* positive (*C. jejuni*). The positive samples (mostly fresh fecal samples) were distributed over three conventional and two extensive outdoor flocks on five farms. The five positive flocks were colonized from the fifth to seventh week of age and remained colonized until slaughter. RFLP yielded five and PFGE six different patterns. Each of the flocks showed one flock-specific genotype predominating until slaughter.

Significance: Presuming different ways of entry, a combination of this fact and the observed dynamics of *C. jejuni* spread within the flocks indicates that a single source from the environment may have been responsible for the colonization of each of the flock. These conclusions may serve to develop combat strategies at farm level.

P2-10 DSC *Salmonella* on Harvest-ready Cattle of the Texas High Plains

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Introduction: *Salmonella* is a pathogen commonly associated with cattle. Multi-drug resistant (MDR) *Salmonella* has become a concern to cattle producers.

Purpose: The objectives of this study were to: (A) identify and characterize specific at-risk populations of cattle most likely to harbor MDR at time of harvest; and (B) determine if prevalence and pathogen load of *Salmonella* varies across season.

Methods: Hide-swab (1,000 cm^2) samples were collected in-plant from three cohorts (cull-dairy, cull-beef, healthy-beef). Fecal samples (~150 g from pen-floor fecal-pats) were collected from the pens of two cohorts in feedlots (culls {(railers)}, healthy harvest-ready). *Salmonella* was isolated using Rapport-Vassiliadis broth, tetrathionate broth and presumptive colonies streaked onto Xylose Lactose Tergitol™ 4 agar. Samples presumptively positive were enumerated using most-probable number (MPN) techniques to provide statistical estimates of colony forming units (reported as MPN/100 cm^2 of hide or MPN/g feces). Isolates were subjected to antimicrobial susceptibility testing using micro-broth dilution techniques and serotyped.

Results: *Salmonella* was recovered from 55.6% of 1,681 samples collected though four seasons. Prevalence on hides was 69.6% and in feces was 30.3%. Prevalence of *Salmonella* on hides of healthy-beef, cull-beef, and cull-dairy was 79%, 58%, and 70%, respectively. Prevalence of *Salmonella* in feces of healthy harvest-ready and cull feedlot cattle was 32% and 28%, respectively. High-level resistance (4+ drugs) was rare. Overall, only 2.2% of all isolates were resistant to four-or-more drugs and was over-represented in isolates recovered from cull cattle. Twenty-two serovars were identified, with the most common being Anatum (25.5%), Montevideo (22.2%), Cerro (12.5%), Mbandaka (10.7%), and Kentucky (7.8%). High-level resistance, which was rare, was most commonly observed in S. Reading.

Significance: *Salmonella* is frequently recovered from healthy and cull cattle in the Texas High Plains. High-level resistance is rare but when it occurs, it is more likely to be detected in isolates recovered from cull animals and serotype clustered.

P2-11 DSC Reduction of *Escherichia coli* O157 and *Salmonella* in Feces and on Hides of Feedlot Cattle Using Various Doses of a Direct-fed Microbial

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Introduction: *Escherichia coli* O157 and *Salmonella* are two pathogens that are commonly associated with healthy feedlot cattle. Direct-fed microbials (DFM) are useful for the reduction of *E. coli* O157 in feces and on hides of market-ready feedlot cattle. To date, the efficacy of DFM on the reduction of *Salmonella* has not been examined.

Purpose: In this study, the effectiveness of DFM at reducing *E. coli* O157 and *Salmonella* in beef cattle was evaluated.

Methods: Steers (n = 240) received one of the following four treatment-concentrations: Control= lactose carrier only; Low = 1×10^7 CFU/steer daily *Lactobacillus acidophilus* NP51; Medium= 5×10^8 CFU/steer daily *L. acidophilus* NP51; and High= 1×10^9 CFU/steer daily *L. acidophilus* NP51. Low, medium and high diets also included 1×10^9 CFU/steer *Propionibacterium freudenreichii* NP24.

Results: Feces was collected from each animal at allocation of treatment and found to have no variation ($P = 0.54$) between cohorts concerning *E. coli* O157 recovery. Feces and hide swabs were collected at harvest and analyzed for the presence of *E. coli* O157, using immunomagnetic separation, and *Salmonella*, using PCR. No significant dietary effects were detected for *E. coli* O157 recovery from feces at the medium-dose or from hides at the medium- and high-doses. *E. coli* O157 was 74% ($P < 0.01$) and 69% ($P < 0.01$) less likely to be recovered in feces from animals receiving the high and low diets, respectively, compared to controls. Compared to controls, *E. coli*

O157 was 74% ($P = 0.05$) less likely to be isolated on hides of cattle receiving the low-dose. No significant dietary effects were detected for *Salmonella* recovery from feces at the medium- and low-doses or from hides at any doses. Compared to controls, *Salmonella* was 48% ($P = 0.09$) less likely to be shed in feces of cattle receiving the high-dose.

Significance: No obvious dose response of *L. acidophilus* NP51 on recovery of *E. coli* O157 or *Salmonella* was detected in the current study.

P2-12 DSC Prevalence and Enumeration of *Escherichia coli* O157 in Steers Receiving Various Strains of *Lactobacillus*-based Direct-fed Microbials; and Validation of Naturally Infected Bovine Feces with a Most-probable Number/Immunomagnetic Separation Technique

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Introduction: *Escherichia coli* O157 is a foodborne pathogen that commonly inhabits the gastrointestinal tract of healthy feedlot cattle. Reduction of this pathogen has become important to producers to insure a safe product for consumers. Direct-fed microbials (DFM) have proven to be effective intervention strategies for reduction of this pathogen in feedlot cattle.

Purpose: The current study had two objectives: (1) to evaluate the effectiveness of daily dietary inclusion of specific strains of *Lactobacillus acidophilus* on prevalence and concentration of *E. coli* O157 in harvest-ready feedlot cattle, and (2) to validate a most-probable number (MPN) / immunomagnetic separation (IMS) technique.

Methods: Steers (n = 500) received one of the following five DFM treatments daily: Control=lactose carrier only; NP28 = 10^9 CFU *L. acidophilus* NP28; NP51 = 10^9 CFU *L. acidophilus* NP51; NP35 = 10^9 CFU *L. acidophilus* NP35; and NP51/NP35 = 10^8 CFU each of *L. acidophilus* NP51 and 35. Immediately before each animal was shipped to the abattoir fecal samples were collected rectally. Selective-enrichment and immunomagnetic separation (IMS) techniques were used to detect *E. coli* O157 in feces. *Escherichia coli* O157 concentration was estimated (culture-positive animals only) using a MPN/IMS technique used for the first time in naturally infected feces.

Results: When compared to controls, steers receiving NP51, NP28, and NP51/NP35 were 57% ($P = 0.02$), 65% ($P < 0.01$), and 65% ($P < 0.01$) less likely to shed *E. coli* O157, respectively. *Escherichia coli* O157 concentration was highest ($P < 0.05$) in controls (3.2 log MPN/g of feces) compared to NP51, NP28, or NP51/NP35 (0.9, 1.1, and 1.7 log MPN/g of feces; respectively).

Significance: Population estimations of *E. coli* O157 were expected using the MPN/IMS technique. Only specific strains of *Lactobacillus*-based DFM effectively reduced the prevalence and concentration of *E. coli* O157 in harvest-ready cattle. Validated strains of DFM should be used to reduce carriage of *E. coli* O157 in feedlot cattle due to the negligible efficacy of certain *Lactobacillus* strains.

P2-13 DSC Impact of In-feed Antimicrobial Drug Use on Antimicrobial Susceptibility Patterns of Generic *Escherichia coli*

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Introduction: The spread of antibiotic resistance has become a leading concern to public health. While expected to play a role in development and spread of antibiotic resistant bacteria, the impact of feeding subtheraputic levels of antimicrobial drugs to feedlot cattle is still highly debated.

Purpose: The purpose of this study was to evaluate the impact of in-feed antimicrobial administration on the populations of generic *E. coli* isolated from feedlot cattle and their susceptibility to common antimicrobial drugs.

Methods: 1,760 animals were allocated to one of 16 pens; each pen was assigned to one of four treatments. Treatments included (a) monensin (29.8 mg/kg dry matter) and tylosin (9.7 mg/kg dry matter); (b) tylosin (9.7 mg/kg); (c) monensin (29.8 mg/kg); and (d) neither of the aforementioned antimicrobials. Fecal samples were collected from 40 animals per pen at arrival, re-implant, and harvest. Hide samples were collected from these same animals prior to shipment. A sub-sample of 10 animals were sampled in the plant post-stunning, post-hide removal and post-intervention. Samples were subjected to culture and enumeration. Multiple isolates collected from each sample were tested for susceptibility to a panel of drugs using a broth microdilution technique. Minimum Inhibitory Concentration breakpoints were obtained from the National Antimicrobial Resistance Monitoring System.

Results: Generic *E. coli* was present in 100% of cattle fecal samples at an average amount of 6.2 log CFU/g. 48.76% of all *E. coli* isolates from samples taken at arrival showed resistance to at least one antimicrobial. Of these resistant isolates, the most common resistance was to Sulfisoxazole, Tetracycline, and Streptomycin with 35.83%, 30.11% and 15.53% showing resistance to the antimicrobials, respectively.

Significance: Analysis of antimicrobial susceptibility patterns of these generic *E. coli* will help identify important risk factors associated with feeding subtheraputic levels of antibiotics to feedlot cattle in the development of bacterial resistance.

P2-14 *Listeria monocytogenes* Growth in Delicatessen Meats Based on Product Formulation and Age

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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. In order to assess the impact of product age on *Listeria* growth, slices of cured turkey breast or ham (25 g) were surface-inoculated with an 8-strain cocktail of *L. monocytogenes* to yield ~40 CFU/g at the time of purchase, midpoint or expiration date, and then pour or surface plated on Modified Oxford Agar to quantify *L. monocytogenes* during storage at 4, 7, and 10°C. All experiments were conducted in duplicate using two different lot numbers of the same product from the same manufacturers. *L. monocytogenes* lag and generation times were then calculated. In general, *L. monocytogenes* grew faster ($P < 0.05$) in deli meats without *Listeria* inhibitors (lactate and/or diacetate) than in those with inhibitors, with the growth rates independent of product age ($P > 0.05$). In turkey breast containing nitrite as the only preservative, growth began after 3 days regardless of storage temperature, with 4, 6 and 9 days of storage at 10, 7 and 4°C respectively required for *L. monocytogenes* to reach potentially hazardous levels of 1000 CFU/g. Generation times for *L. monocytogenes* in cured turkey were 9, 14, and 18 h at 10, 7 and 4°C, respectively. In ham containing lactate and diacetate, growth began after 4, 6 and 9 days of storage at 10, 7, and 4°C, respectively, with 7–8, 11–12, and 15–21 days needed to obtain populations of 1000 CFU/g, respectively. Generation times for *L. monocytogenes* in ham were 13, 22 and 30 h at 10, 7 and 4°C, respectively. Lactate and diacetate prolonged the shelf life of ham, but were only partially inhibitory to *Listeria*. These findings will be useful in developing scientifically based "best consumed by" dating for deli meats.

P2-15 Validation of Hot Water Interventions on Beef Carcasses Using Fluorescent Protein-marked Nonpathogenic *Escherichia coli* Strains as Surrogates for *E. coli* O157:H7 and *Salmonella*

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Hot water carcass washes are currently used extensively in the beef processing industry as pathogen interventions, and while effectiveness is assumed, it has often not been strongly validated. Nonpathogenic *E. coli* strains could be used as surrogates in validation of hot water interventions when pathogens cannot be utilized in food processing establishments. The purpose of this study was to evaluate nonpathogenic *E. coli* strains for use as surrogates in validating hot water treatments and determining temperature–time parameters to achieve maximal reductions on beef carcasses. Fluorescent protein-marked nonpathogenic *E. coli* strains and rifampicin-resistant mutants of *E. coli* O157:H7 and *S.* Typhimurium were used as marker organisms. The surface of hot-boned outside rounds was inoculated with fecal slurry containing the marker organisms. Each outside round was then sectioned in five pieces (300-cm^2 approximately) and each piece randomly assigned to a treatment: (1) water at 76 ± 1°C for 2, 4, 6, 8 or 10 s; (2) water at 86 ± 1°C for 2, 3, 5, 7 or 9 s and (3)water at 97 ± 1°C for 1, 2, 3, 4 or 5 s. Treatments were applied at 30 psi from a 10-cm distance. Two 10-cm^2 excised samples were collected in duplicate for enumeration of marker organisms. Death curves were constructed and D-values calculated from the linear regression equations. D-values among surrogates, *E. coli* O157:H7 and *S.* Typhimurium were not different ($P > 0.05$). The average time required to reduce surrogates and pathogens by a 5-log cycle was 25.0 ± 7, 13.3 ± 3 and 9.6 ± 3 s when the temperature on the carcass surface was 70.9 ± 2, 78.5 ± 2 and 86.4 ± 2°C respectively. Results indicate that the fluorescent protein-marked *E. coli* strains used in this study are appropriate surrogates for validating hot water interventions for pathogen reduction on beef carcasses.

P2-16 Investigation of a Multi-step Intervention suitable for Very Small Meat Establishments to Reduce Pathogens from Inoculated Beef Surfaces

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Given the financial, space, and labor constraints of very small meat establishments (VSME), it is necessary to identify low-cost, practical antimicrobial carcass treatments that meet VSME needs. Multi-step treatments, comprising separate steps for cleaning and sanitizing, may be more efficacious than one-step treatments currently employed by the meat industry. In this study, one-step and multi-step antimicrobial treatments were investigated to determine optimal microbial decontamination of beef surfaces. Beef brisket surfaces were inoculated with a fecal slurry containing *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter coli*, and *C. jejuni*. Treatments consisted of a warm (54°C) water wash only (W0), warm water wash plus water rinse (WW), water rinse only (0W), warm water wash plus 2% lactic acid rinse (WA), or a 2% lactic acid rinse only (0A). Water washes were applied using a packinghouse hose (30 psi, 20 s, distance < 5 cm), allowed to drip for 5 min, with the lactic acid applied using one of four different hand-held sprayers (stainless steel, backpack, garden, or retrofitted garden; 40 psi, 15 s, distance < 5 cm). Following treatment, beef briskets surfaces were processed for enumeration and isolation of pathogens and hygiene indicators (mesophilic aerobic plate counts, coliforms, and generic *E. coli*) and log reductions determined. Regardless of the spray apparatus used, WA was the most effective treatment for reducing bacterial populations (2.38 to 8.86 log CFU/cm^2). While 0A reduced populations (0.58 to 5.06 log CFU/cm^2) significantly, the reduction of microbial populations following water-only treatments ranged from – 0.80 to 3.24 log CFU/cm^2. Use of a stainless steel tank yielded at least 3.68 and 4.40 log CFU/cm^2 greater reductions ($P = 0.002$) of mesophilic APC and coliforms, respectively, than the other spray tanks

investigated. The WA treatment, using a stainless steel tank, will be used for further studies to validate its use as a multi-step antimicrobial carcass intervention in VSME.

P2-17 Investigation of Antimicrobial Rinses Suitable for Very Small Meat Establishments to Reduce Pathogens from Inoculated Beef Surfaces

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Numerous antimicrobial interventions are capable of reducing the prevalence of harmful bacteria on raw meat products. Because of limited labor, space, and financial resources, there is a need to identify effective and inexpensive antimicrobial interventions that are feasible for use in very small meat establishments and can be integrated into hazard analysis and critical control point (HACCP) programs. The bactericidal effectiveness of eight antimicrobial compounds (acetic acid, citric acid, lactic acid, peroxyacetic acid, acidified sodium chlorite, chlorine dioxide, sodium hypochlorite, and aqueous ozone) at various concentrations was examined. Beef brisket pieces were inoculated with a fecal slurry containing a six-strain pathogen cocktail (*Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter coli*, and *C. jejuni*). Antimicrobial solutions were applied to beef surfaces via a portable, pressurized, stainless steel spray tank (40 psi, distance of 30 cm from spray nozzle to meat surface, 15 s, 5 min dwell time). Treated surfaces were subjected to appropriate methods for the enumeration and isolation of pathogens and hygiene indicators (mesophilic aerobic plate counts, coliforms and generic *E. coli*). Antimicrobial effectiveness was determined as follows: organic acids > peroxyacetic acid > chlorinated compounds > aqueous ozone. For example, a 2% lactic acid rinse provided log reductions ranging from 3.5 to 6.4 log CFU/cm^2 across all bacterial populations studied. Conversely, aqueous ozone yielded 0.02 to 2.9 log CFU/cm^2 reductions in pathogens and hygiene indicators and did not differ significantly from a control tap water rinse (P-values = 0.055 to 0.731). Additional experiments will employ the 2% lactic acid rinse described here as part of a multi-step antimicrobial intervention under laboratory conditions, as well as part of an in-plant validation study for very small meat establishments.

P2-18 Control of *Listeria monocytogenes* and Other Microorganisms in Brine Chill Systems Using a Novel Antimicrobial Formulation

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Introduction: Brine chillers are potential risk areas for contamination of food by pathogens. *Listeria monocytogenes*, for example, survives well in saturated salt brines at cold temperatures and is resistant to commonly used hypochlorite brine treatments.

Purpose: The purpose of this study was to explore new, food-safe, non-acidic antimicrobial additives for use in food production brines.

Methods: Brines containing 24% NaCl and various surfactants were inoculated with cocktails containing approximately equal numbers of 5 strains of *L. monocytogenes*, to a level of approximately 10^6 CFU/ml and incubated at -18.5°C +/- 2°C. *L. monocytogenes* populations were determined at various time intervals by plating to Modified Oxford Agar with a Trypticase Soy Agar thin-agar overlay to aid in recovery of injured cells. Biofilms of this same cocktail were grown on stainless steel coupons, challenged with brine formulas, and enumerated as described above.

Results: A 25 ppm (final concentration) solution of an alcohol ethoxylate in freshly prepared brine showed an ~ 3 log reduction in *L. monocytogenes* after 15 min and an ~ 6 log reduction after 30 min. Spent chill brine from a commercial hot dog production line containing 50 ppm (final concentration) of an alcohol ethoxylate reduced the inoculated *L. monocytogenes* cocktail to undetectable levels (<10 CFU/ml) within 4 h. When *L. monocytogenes* biofilm was treated with spent chill brine containing 50 ppm alcohol ethoxylate, counts decreased to 10–100 CFU/coupon within 1 h compared to 44,000 CFU/coupon for the control.

Significance: Certain low toxicity surfactants, at relatively low concentrations, produced a strong synergistic antimicrobial effect with salts commonly used in brine chilling systems. These novel, patented formulas retain antimicrobial activity in the presence of organic material and are effective against *L. monocytogenes* biofilms, making them a potentially valuable tool for enhancing the safety of many chilled food products.

P2-19 DSC Reduction of *Escherichia coli* O157:H7 in Whole Muscle Beef Cuts Using Lactic Acid Bacteria Cultures

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Introduction: A commercially available antimicrobial product contains four strains of lactic acid bacteria (LAB), and is a post-production treatment used in the significant reduction of foodborne pathogens in beef products. The mixture has passed GRAS (Generally Recognized as Safe) status assessment by the FDA, and is one of few post-production treatments available to protect meat during long-term storage, without affecting the shelf life or flavor.

Purpose: The intent of this study was to evaluate the reduction of *E. coli* O157:H7 on chilled beef trim and on warm beef surfaces after inoculation with LAB cultures.

Methods: Beef briskets were obtained for two distinct studies; warm briskets (37°C) simulated inoculation of beef carcasses, which were cooled to 5°C to simulate chilling, and chilled briskets (5°C) cut into pieces that simulated beef trim. The briskets were inoculated with a 10^7 CFU/cm^2 dose of a four-strain cocktail mixture of *E. coli* O157; then half of the

briskets were inoculated with a 10^7 CFU/cm^2 dose of LAB cultures while the other half were treated with sterile water. Samples were collected at 0, 2, 4, 12, 24, 48, 72 and 96 h to determine reductions of *E. coli* O157 on the surfaces of the meat.

Results: Significant reductions (1.5 log cycles) of *E. coli* O157 occurred 2 h after application of LAB cultures in the warm brisket, and significant reductions (2.0 log cycles) occurred after 6 h for the chilled brisket, with slight reductions in pathogen loads occurring after 48, 72 and 96 h in both studies. The total LAB counts were similar between the two treatments after 48 h of storage, indicating that the total counts of LAB were not changed, just the composition of the type of LAB found on the product. In all samples, there were no differences in total psychrotrophic organisms between the treated and non-treated samples, while the total populations of LAB in the non-treated samples increased over time.

Significance: The data indicates that when this combination of lactic acid bacteria cultures are added to hot carcasses, primal or subprimal cuts, or to trim in beef, significant reductions of pathogen loads occur. Additional reductions do not occur after the first 4–6 h of application; thus there is no residual effect because the surface environment inactivates some of the lactic acid bacteria, and the lower amounts of total bacteria added result in less of a killing effect.

P2-20 Survival of *Listeria monocytogenes* Inoculated Post-processing on Frankfurters Formulated with Low Concentrations of Malic Acid, Sodium Citrate, and Sodium Acetate, and Exposed to a Heat Treatment after Packaging

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Introduction: Ready-to-Eat meat products may become contaminated with *Listeria monocytogenes* after thermal processing and, thus, there is a need to develop chemical and/or physical hurdle technologies to enhance the safety of these products.

Purpose: This study evaluated the antilisterial effects of antimicrobials added at low concentrations to the formulation of frankfurters, with and without application of a post-packaging heat treatment.

Methods: Frankfurters (two replications; three samples each) formulated with no antimicrobials (control), malic acid (MA; 0.032% wt/wt), sodium citrate (SC; 0.06% wt/wt), sodium acetate (SA; 0.12% wt/wt), or MA+SC+SA were inoculated (4 log CFU/cm^2) with a 10-strain mixture of *L. monocytogenes* and vacuum-packaged (one frankfurter/bag). Inoculated and vacuum-packaged control and MA+SC+SA frankfurters were also immersed into boiling water (93 ± 2°C; 5 or 15 s). Samples were stored (7°C, 60 days) and periodically analyzed for *L. monocytogenes* (PALCAM agar) and total bacterial (tryptic soy agar plus 0.6% yeast extract) populations.

Results: Initial (day-0) pH values of all product formulations were similar (5.89 ± 0.11 to 6.04 ± 0.05). The post-packaging heat treatments reduced initial pathogen populations by 1.2–1.4 log CFU/cm^2 (5 s) and 1.8–2.0 log CFU/cm^2 (15 s). During storage, growth of *L. monocytogenes* was not different on control, MA and SC samples, and populations exceeded 7.0 log CFU/cm^2 by day-19. In contrast, growth on SA and MA+SC+SA samples was inhibited, and levels were 1–2 log CFU/cm^2 lower than the other formulation treatments on day-19. Compared to non-treated samples, post-packaging heat treatments extended the lag phase of *L. monocytogenes* on control and MA+SC+SA samples, with better effects obtained for samples treated for 15 s. Inclusion of MA+SC+SA in the formulation, in combination with the initial reductions resulting from the heat treatment (5 or 15 s), allowed increases of the pathogen of <1.0 log CFU/cm^2 in 19 or 25 days.

Significance: Findings of this study could be useful to US meat processors in their efforts to control post-processing contamination of frankfurters with *L. monocytogenes*.

P2-21 DSC Acetate, Lactate Levels in Ready-to-Eat Processed Meat and Poultry Products Collected at Retail and Correlation with Occurrence of *Listeria monocytogenes*

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Listeria monocytogenes (Lm) is considered a serious foodborne pathogen that has been isolated mostly from Ready-to-Eat (RTE) processed deli meat and poultry products. Contaminated food products are responsible for approximately 2000 cases of listeriosis in the US each year. The purpose of this study was to quantify the levels of lactate, and acetate (organic acids) occurring in retail RTE processed deli meat and poultry products to determine the impact of current antimicrobial lethality treatments on occurrence of LM at retail. Pre-packaged and deli meat and poultry luncheon meats samples (2000 samples) were randomly selected out of 8000 samples previously analyzed for LM and were collected from four FoodNet states (TN, GA, CA, and MN). Lactic acid and acetic acid were extracted and then analyzed on HPX 87H ion exclusion column (BioRad, Hercules, CA) using HPLC system with PDA detector (Dionex, Sunnyvale, CA). Mobile phase was 5 mM H_2SO_4, flow rate 0.60 ml/min, injection volume 10 l, and absorbance was monitored at 210 nm. Levels of lactic acids in Tennessee samples (500 samples) ranged from 1.06 to 35.4 mg/g. The largest number of samples (27.2%) had lactic acid levels from 10–15 mg/g, followed by 26% of the samples having 5–10 mg/g. Acetic acid ranged from 0.01 to 17.2 mg/gm and was not found in about 8% of the samples. Positive LM samples (17 samples) had lactic acid levels from 3.8 – 24 mg/g and

acetic acid levels ranging from 0.22–5.7 mg/g. Based on these data, there did not appear to be a relationship between residual levels of lactate or acetate in RTE meat and poultry and occurrence of Lm.

P2-22 Use of Buffered Sodium Citrate and Sodium Diacetate to Control *Listeria monocytogenes* on Hams

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Control of *Listeria monocytogenes* on Ready-to-Eat meat products during refrigerated storage can minimize the risk of foodborne illness from this pathogen. Currently, sodium lactate in combination with sodium diacetate is widely used to control *L. monocytogenes* growth. Identification of additional antimicrobials that minimize the undesirable quality effects will help the industry control the pathogen as well as preserve product quality. Effectiveness of buffered sodium citrate and sodium lactate in combination with sodium diacetate (0.2%) at 0.8, 1.6 and 2.4% concentrations to control *L. monocytogenes* growth under refrigeration was evaluated. Hams were manufactured using a traditional formulation to include buffered sodium citrate or sodium lactate in combination with sodium diacetate at 0.8, 1.6 and 2.4%, along with two controls (no antimicrobial, with sodium diacetate, 0.2%). Ham surfaces were inoculated with a five-strain cocktail of *L. monocytogenes* to attain ca. 1.5 CFU/cm^2, vacuum packaged and stored (4°C or 10°C), and sampled at one or 2 week intervals. *L. monocytogenes* populations increased by >2.0 log within 4 and 8 weeks at 4°C for control and product with sodium diacetate alone. Incorporation of buffered sodium citrate at 0.8% in addition to sodium diacetate extended the time required for 2 log increase to 10 weeks. For all other concentrations of buffered sodium citrate and sodium lactate, less than 2 log increases of *L. monocytogenes* populations were observed throughout the 18-week storage period. At abusive temperate (10°C), greater inhibition was observed by increasing the concentrations of the antimicrobials. While sodium lactate in combination with sodium diacetate is currently being used in Ready-to-Eat meat products, buffered sodium citrate can also be used as an antimicrobial to control *L. monocytogenes*.

P2-23 Fate of *Listeria monocytogenes* in a Processed Meat with Sodium Lactate and Diacetate and a Biopreservative

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To determine the impact of the combination of sodium lactate, diacetate and a biopreservative on the fate of *Listeria monocytogenes*, three separate batches of a simulated Ready-to-Eat wiener were manufactured in a chubb formulation in a pilot plant. The inhibitory agents were added to the formulation prior to processing. The biopreservative Micocin® (*Carnobacterium maltaromaticum* CB1) was added at 0.01, 0.05, 0.1, 0.5, 1 and 5% and the concentration of sodium lactate/diacetate mixture (chemical) was used as per industry standards. After processing, the chubbs were surface inoculated to give approximately 1 x 10^3 of a cocktail of *L. monocytogenes* (including serotypes 1/2a, 1/2b, 3a and 4b) per gram of the sliced product. The inoculated slices were vacuum-packaged, stored at 4°C and *Listeria* were enumerated on PALCAM agar during a 10-week storage period. In the control samples without the chemical or the biopreservative, *Listeria* spp. increased to a population of 10^9 CFU/g within 35 days. In contrast, in the presence of the chemical or biopreservative the *Listeria* grew slower than on the control but eventually reached 10^8 CFU/g within 70 days of storage. In the product with both chemical and Micocin® (minimum of 0.5%), an initial decrease below the inoculum level was achieved and counts were maintained below the inoculum level throughout the 10-week storage period. The combination of the Micocin® biopreservative and chemical antimicrobials in the meat batter prior to processing could allow meat processors to implement Alternative 1 of the USDA/FDA criterion for control of *L. monocytogenes* in Ready-to-Eat meats.

P2-24 Viability of *Listeria monocytogenes* on Commercially-prepared Roast Beef Log, Turkey Breast Log, and Frankfurters Surface Treated with Lauric Arginate and Stored at 4°C for 24 Hours

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Introduction: *Listeria monocytogenes* is the causative agent of listeriosis, a serious disease that can cause death in susceptible populations. This disease is most commonly associated with Ready-to-Eat food products which support the growth of the pathogen and are consumed without additional cooking. As such, the presence of *L. monocytogenes* in these food products is strictly regulated by federal agencies.

Purpose: Evaluate the effectiveness of the Sprayed Lethality In Container (SLIC) system to deliver an antimicrobial for lowering the levels of *L. monocytogenes* on the surface of roast beef log, turkey breast log, and frankfurters.

Methods: Each of the meat products were surface inoculated with a five-strain cocktail of *L. monocytogenes* (7.0 log CFU) and added to packages containing various volumes (0 to 6 ml) and concentrations (5 or 10% in dH_2O) of lauric arginate (LAE; Ethyl-N-dodecanoyl-L-arginate hydrochloride). Each package was vacuum-sealed and stored at 4°C for 24 h.

Results: Pathogen levels decreased by ca. 1.0 to 2.0 log CFU/package in roast beef samples treated with 4 or 6 ml of 5% or 10% LAE. In turkey breast samples, using 4 or 6 mL, pathogen levels decreased by ca. 1.5 to 3 log CFU/package with 5% LAE and ca. 4.5 log CFU/package with 10% LAE. In frankfurters pathogen levels decreased by ca. 3.5 log CFU/package with 2 mL

of 5% or 10% LAE and ca. 5 log CFU/package in frankfurter samples treated with 4 or 6 mL of either a 5% or a 10% solution of LAE.

Significance: These results show that application of LAE via the SLIC method reduced *L. monocytogenes* levels on the surfaces of roast beef, turkey breast and frankfurters within a 24 hour period at 4°C. The application of LAE as a post-process intervention should assist manufacturers in achieving the USDA/FSIS alternative 2 status.

P2-25 Antimicrobial Effects of A&B Ingredients CytoGuard® on the Survival of *Listeria monocytogenes* Surface-inoculated onto Bar-S® Foods Co. Hot Dogs, Vacuum-packed and Stored at 4°C

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Listeria monocytogenes, with its ubiquitous nature and its pathogenic characteristics, presents a unique dilemma in post-processing, pre-packaging contamination of Ready-to-Eat (RTE) food products, such as hot dogs. This study evaluated the ability of A&B Ingredients, Inc. CytoGuard® LA (lauric arginate) to reduce and/or eliminate the presence of *Listeria monocytogenes* on surface-inoculated Bar-S® hot dogs. A six-strain "cocktail" of *L. monocytogenes* (10^{4-5} CFU/ml) was used for surface inoculation onto commercially prepared hot dogs (16 oz package), processed without an antimicrobial agent. The packages were opened aseptically and the hot dogs were carefully slide into vacuum pouches (Prime Source®), being sure to transfer the purge as well as hot dogs. One ml aliquots of inocula were added to each pouch and massaged for 2 min. After inoculation, 3 ml of either 5% Cyto-Guard® LA (diluted by the addition of sterile dd water), or 0.1% sterile peptone water was added to the pouches. Samples were immediately vacuum-sealed at -100 kPa with a Koch's Ultravac and stored at 40°F for 0, 12, 24 and 72 h. Time 0 was processed immediately after treatment. Serial dilutions were plated onto MOX agar and incubated at 37°C for 72 h to allow for recovery of damaged cells. These results indicated that CytoGuard®, at the test dilution, caused a 3.33, 2.14, 2.52 and 2.12 log reductions of *L. moncytogenes* at 0 h, 12 h, 24 h and 72 h, respectively. These findings are important for RTE hot dog processors to further assure the safety of their products to consumers.

P2-26 Effect of Combining Nisin and/or Lysozyme with In-package Pasteurization for Control of *Listeria monocytogenes* in Ready-to-Eat Turkey Bologna during Refrigerated Storage

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Post-processing contamination of Ready-to-Eat (RTE) meat and poultry products by *Listeria monocytogenes* is a major food safety issue. Because of the ability of *L. monocytogenes* to grow and multiply at refrigeration temperatures, even a low level of initial contamination can result in a substantial number on the food product at the time of consumption. This study investigated the efficacy of in-package pasteurization combined with pre-surface application of nisin and/or lysozyme to reduce and prevent the subsequent recovery and growth of *L. monocytogenes* during refrigerated storage on the surface of low fat turkey bologna. Sterile bologna samples were treated with solutions of nisin (2 mg/ml = 5000 AU/ml), lysozyme (10 mg/ml = 80 AU/ml) and a mixture of nisin and lysozyme (2 mg of nisin + 10 mg of lysozyme/ml). Bologna surfaces were uniformly inoculated with a *L. monocytogenes* suspension resulting in a population of 8 log CFU/slice. Samples were vacuum-packaged and subjected to in-package pasteurization at 65°C for 32 s and stored at 4°C for 12 weeks. In-package pasteurization resulted in an immediate 3.5–4.2 log CFU/cm^2 reduction in *L. monocytogenes* for all treatments. *L. monocytogenes* population remained fairly constant on all non-pasteurized bologna treatments throughout 12 weeks of refrigerated storage. In-package pasteurization of control (no antimicrobial treatment) and lysozyme treatments resulted in a reduction of 2.4 log CFU/ cm^2 by 12 weeks and 1.9 log CFU/cm^2 by 8 weeks, respectively. Nisin and nisin-lysozyme treatments in combination with in-package pasteurization were effective in reducing the bacterial population to below detectable levels by 2–3 weeks of storage. Results from this study could have a significant impact for the industry since the reduction in bacterial population was achieved by a relatively short pasteurization time and antimicrobials reduced the population further during refrigerated storage.

P2-27 Efficacy of SANOVA RTE in Reducing *Listeria monocytogenes* Populations in Ready-to-Eat Products

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Introduction: *Listeria monocytogenes* is an organism of great concern to the Ready-to-Eat (RTE) meat industry due to its propensity to persist in typical plant environments. Possible post-cooking contamination of these products prior to packaging and distribution can expose consumers to serious illness or even death. Ecolab has developed the SANOVA RTE treatment as a post-lethality intervention on both whole and sliced products to reduce and/or eliminate this pathogen on RTE products.

Purpose: The purpose of this study was to investigate the ability of SANOVA RTE to reduce or eliminate *Listeria monocytogenes* inoculated onto the surface of whole RTE products.

Methods: A multi-strain cocktail of *Listeria monocytogenes* was surface inoculated onto several different

types of RTE products (cured turkey deli log, uncured turkey deli log, cured pork ham, uncured pork ham, cured bologna, uncured turkey bacon, uncured roast beef, and uncured beef pastrami). After allowing time for bacterial attachment, products were treated with SANOVA Food Quality system. After 2 h of refrigerated storage, sliced samples were rinsed with UVM broth and the rinsate was plated onto MOX plates. *Listeria monocytogenes* amounts remaining on treated samples were compared to matching control samples to determine the reduction produced by SANOVA RTE.

Results: On average, for all products examined, *Listeria monocytogenes* was reduced by 2.49 log CFU/ml of rinsate. The largest reduction (2.81 log) was observed in bologna, while the least reduction (2.26 log) was observed in cured turkey.

Significance: This data indicates that treatment with SANOVA RTE reduces *Listeria monocytogenes* present on the surface of several types of RTE products by greater than 99.0%. This intervention therefore represents an effective addition to the post-lethality interventions in use by an RTE processing facility.

P2-28 Inhibition of *Listeria monocytogenes* by Meat-borne Spoilage Lactic Acid Bacteria and *Bacillus* Species

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Introduction: *Listeria monocytogenes* is a pathogen that is of concern for Ready-to-Eat (RTE) meats because it can tolerate high concentrations of salt and sodium nitrite and grows at refrigerated temperatures. Recently, sodium diacetate and sodium lactate have been added to RTE meats to inhibit *L. monocytogenes*; however, they can produce flavor defects. Use of protective cultures and bacteriocins to inhibit the growth of *L. monocytogenes* may be an alternative.

Purpose: The purpose of this study was to examine the effect of spoilage microflora, *Bacillus* spp. and lactic acid bacteria (LAB) on the growth of *L. monocytogenes* at low inoculum levels.

Methods: Twenty-eight strains each of LAB and *Bacillus* spp., isolated from Ready-to-Eat deli ham, were screened by agar spot assays for their inhibition toward growth of *L. monocytogenes*. LAB and *Bacillus* strains that showed the greatest inhibition toward *L. monocytogenes* were tested for their action against eleven additional strains at various concentration levels and their sensitivity to proteinase K treatment. LAB isolates were tested under conditions preventing the production of lactic acid to determine the mechanism of inhibition.

Results: Two LAB strains showed the same level of inhibition against *L. monocytogenes* whether at 10^2 – 10^3 or 10^6–10^7 CFU/ml and one LAB showed lower activity if no lactic acid was produced; however, *Bacillus* spp. were not as effective at lower inoculum levels as were the LAB. Three LAB strains inhibited all eleven *L. monocytogenes* indicator strains; whereas only one *Bacillus* isolate did and another *Bacillus* isolate inhibited seven of the eleven *L. monocytogenes* indicator strains. One LAB isolate and one *Bacillus* isolate showed sensitivity to proteinase K treatment, indicating possible bacteriocin production with the *Bacillus* cell free suspension also producing inhibition.

Significance: Based on these results, a preservative that controls the growth of *L. monocytogenes* could be developed.

P2-29 Thermal Inactivation of High Pathogenicity Avian Influenza Viruses in Chicken Meat

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High pathogenicity avian influenza (HPAI) viruses cause severe disease with high mortality in chickens and related gallinaceous poultry. Some HPAI viruses cause systemic infections and replicate to high titers in skeletal muscle fibers. To prevent transmission of these viruses though contaminated meat, the World Organization for Animal Health (OIE) recommends trading poultry products from countries, zones, or compartments infected with avian influenza subtypes H5 or H7 only when the products are effectively processed to destroy these pathogens. To determine thermal inactivation parameters for HPAI in chicken meat, thermal inactivation of the HPAI strains A/chicken/Korea/ES/2003 (H5N1) and A/chicken/PA/1370/83 (H5N2) was quantitatively measured in meat harvested from infected chickens. Small (0.5 g) pieces of meat were placed in thin-walled PCR tubes and heated in a thermocycler block. After heat treatment, the meat samples were ground with small pestles to release virus from the tissue, and the supernatants were tested for virus inactivation by titration in embryonating chicken eggs. The times required for a 90% reduction in virus titer (D-values) were calculated for each HPAI strain at temperatures ranging from 57°C to 61°C in 1°C intervals, and line equations describing the thermal inactivation of each HPAI strain were generated from graphs of D-value (log scale) versus temperature. Similar D-values were predicted by the line equations for each HPAI strain at each temperature tested. However, the temperature increase needed to reduce the D-value by 90% (z-value) for the H5N2 strain (4.1°C) was 0.5°C lower than that for the H5N1 strain, indicating that a slightly higher sensitivity to increasing temperature was observed with the H5N2 samples. Each line equation predicts that cooking chicken meat according to current USDA-FSIS time-temperature guidelines for a 7-log reduction of *Salmonella* will inactivate HPAI in a heavily contaminated meat sample with a large margin of safety.

P2-30 Antimicrobial Effects of Mastertaste Zesti-smoke (Two-stage Application) on the Survival of *Listeria monocytogenes*, Surface-inoculated onto Bar-S Hot Dogs, Vacuum-packed and Stored at 4°C

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Post processing, pre-packaging contamination of hot dogs presents a complex problem to Ready-to-Eat (RTE) food processors. This study evaluated the ability of three Mastertaste products to reduce and/or eliminate the growth of *Listeria monocytogenes* on surface-inoculated Bar-S® hot dogs. A six-strain "cocktail" of *L. monocytogenes* (10^{3-4} CFU/ml) was used as inoculum. Commercially prepared 16-oz packaged hot dogs, processed without antimicrobial agent, were placed in vacuum pouches (Prime Source®). One ml aliquots of inoculum were added to each sample and massaged for 2 min. The contents of each pouch were placed onto a polystyrene weigh-dish, previously sprayed with 1.5 ml Mastertaste Zesti-Smoke products (90% AM3, NaturSaf 99.3, NaturSaf 99.4), or sterile dd water (control). Another 1.5 ml of the product (or water) was sprayed on top of the samples using an AutoJet® Technologies, Model 2250 Spray Controller with a PulsaJet® nozzle. Samples were vacuum-sealed and stored at 40°F for 12, 18, 24, 36, and 48 h. Time 0 was processed immediately after spray treatment. Serial dilutions were plated onto MOX agar and incubated at 37°C for 72 h. At 12 h of storage, 90% AM3 caused a 1.06 log reduction of *L. monocytogenes*, while NaturSaf 99.3 caused a 1.04 log reduction at 18 h and NaturSaf 99.4 caused a 1.03 log reduction at 24 h. These results are important for RTE hot dog processors to further assure the safety of their products to consumers.

P2-31 DSC Ceftiofur Crystalline-free Acid Administration Reduces Susceptibility of Generic *Escherichia coli* in Cattle

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The objective of the study was to determine the extent and duration of effects of ceftiofur crystalline free acid (CCFA) administered on antimicrobial drug susceptibility of generic *E. coli* in feedlot cattle. A cohort study was conducted using 61 feedlot cattle housed in 6 10-head pens (1 pen contained 11). Five head in each pen were exposed and 5 served as controls (1 pen contained 6 controls). Ceftiofur crystalline free acid exposures included 6.6 mg/kg BW ceftiofur once on day 0 (single-dose regimen), 6.6mg/kg on days 0, 6, and 13 (3-dose regimen), and 4.4 mg/kg once on d0 (2/3-dose regimen). Fecal samples were collected on days 0, 2, 6, 9, 13, 16, 20, and 28. Minimum inhibitory concentrations of a panel of antimicrobial drugs were determined using broth micro-dilution for 3 well-defined colonies per sample. Additionally, direct-plating on MacConkey's agar was used to enumerate *E. coli* in each sample. Of the 1,441 isolates recovered 68.1% were resistant to 1 or more antimicrobial drugs. Exposure to CCFA, regardless of regimen, was associated with a transient increased in likelihood of recovery and expansion of the population of ceftiofur (Cef)-resistant isolates ($P < 0.1$ for all models). Populations of *E. coli*, regardless of exposure regimen, returned to baseline approximately 2 weeks post CCFA administration. Almost perfect agreement between resistance to Cef and co-resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfisoxazole (Su), and tetracycline (T) was observed (Kappa = 0.97). Twenty percent of all isolates displayed the ACSSuT+Cef phenotype and also exhibited reduced susceptibility to ceftriaxone (≥ 2 μg/mL). Variation in *E. coli* populations of commingled controls was not detected. Ceftiofur crystalline free acid provided a transient selection pressure favoring expansion of resistant variants. However, resistance appears to exert a fitness cost and pre-exposure 'levels' of resistance were reestablished soon after the selection pressure was removed.

P2-32 Combined Effect of Formulation and High-pressure Pasteurization on the Control of *Listeria monocytogenes* on Ham

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Multiple hurdles of formulation and high-pressure pasteurization (HPP) treatments were evaluated on the inactivation and growth suppression of *Listeria monocytogenes* (Lm) on ham stored under mild temperature abuse conditions (7°C). Trial 1 ham contained 69% moisture and 1.5% NaCl, whereas Trial 2 contained 73% moisture and 2.1% NaCl; within each trial, ham was formulated with either 2.9 or 3.2% commercial lactate-diacetate blend (LD). Ham slices were surface inoculated with 5 log CFU Lm (5-strain mixture) per 100 g package, packaged under modified atmosphere, HPP treated at 580 MPa, 21°C, for 3 or 4 min, and then stored at 7°C for up to 24 weeks; triplicate samples per trial were assayed at each sampling interval. HPP treatments inactivated >3 and 1.1 log Lm on ham for Trials 1 and 2, respectively. Within a given trial, there was no significant difference ($P < 0.05$) in initial inactivation between the two lactate-diacetate levels or the minutes of treatment. Ham formulated with 2.9% LD and treated 3 or 4 minutes with HPP prevented recovery and growth for 10 weeks at 7°C, but populations of Lm increased by >1 log at 12 weeks. Ham formulated with 3.2% LD and treated 3 min with HPP suppressed growth for 12 weeks, but supported >1 log growth at 14 weeks. In contrast, populations of Lm reduced to <1 log CFU per package at 8 weeks for both trials of the 3.2% LD-4 min HPP treatment; populations remained at <1 log CFU per package for the duration of the 7°C storage, regardless of initial log reduction affected by the HPP treatment. This study demonstrates that combining the multiple barriers of 3.2% lactate-diacetate and 4 min HPP will enhance the safety of Ready-to-Eat meats when exposed to mild temperature abuse by inhibiting the recovery of injured cells.

P2-33 DSC Ultraviolet Light Dose Required to Inactivate *Listeria monocytogenes* in Water and Brine

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Ultraviolet light (UV) may be an effective tool for control of *Listeria monocytogenes* in brines used to cool Ready-to-Eat (RTE) meat products. The purpose of this research was to establish the minimum dose of UV (peak: 253.7nm) required to inactivate *L. monocytogenes* in water and a 9% NaCl solution using uridine as a chemical actinometer. *L. monocytogenes* strains N1-227 (hot dog batter), N3-031 (turkey franks), and R2-499 (RTE meat) were mixed in equal proportions 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 irradiated for 1, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, or 120 min using an Oriel photoreactor (model 66901) fitted with a filter to allow only UV light in the 253.7 +/- 10 nm range to pass to the sample. The sample was held at 8°C (+/-2°C) and continuously stirred during UV exposure. Inactivation was evaluated by serially diluting samples in 0.1% peptone, surface plating onto Modified Oxford Agar (MOX) and Trypticase Soy Agar with Yeast Extract (TSAYE), and by enrichment in Brain Heart Infusion Broth (BHI), followed by incubation at 35°C for 24 h. The absorbance of each sample was measured before and after irradiation, using a Shimadzu spectrophotometer (model UV-2101PC). *L. monocytogenes* irradiated in water decreased to below the detection limit (1 log CFU/ml) at UV doses greater than 23 mJ/cm^2, but was detected via enrichment after exposure of up to 95 mJ/cm^2. *L. monocytogenes* irradiated in 9% salt decreased to below the detection limit after exposure to UV in the 28 and 54 mJ/cm^2 range, but was detected via enrichment after exposure to UV at doses greater than 54 mJ/cm^2. Knowledge of UV dose required to control *L. monocytogenes* in brines used during RTE meat processing will aid manufacturers in establishing appropriate food safety interventions for these products.

P2-34 Control of Foodborne Pathogens in Ground Beef Using Controlled Phase Carbon Dioxide

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Decontamination of beef trimmings is a challenging task in the beef industry because ground beef is considered a single ingredient. Controlled phase carbon dioxide ($_{CP}CO_2$) may effectively control spoilage and pathogenic microorganisms on trimmings with no detrimental effects in finished ground beef. The effectiveness of $_{CP}CO_2$ applied on beef trimmings as antimicrobial intervention for ground beef and its effects on organoleptic characteristics of raw and cooked ground beef under different packaging atmospheres was evaluated. Beef trimmings challenged with generic *E. coli* (GEC), *Escherichia coli* O157:H7 (EC), and *Salmonella* spp. (SS) exposed to 1500 and 750 psi $_{CP}CO_2$ at 36°C for 5 and 15 min. Bacterial reductions on beef trimmings and ground beef were analyzed using the Thin Agar Layer method with MacConkey Sorbitol Agar for GEC and EC, and Xylose Desoxycholate Agar for SS. Patties obtained from non-challenged treated beef trimmings were packaged on Styrofoam trays with PVC overwrap, a subgroup was additionally flushed with 100% CO_2. Instrumental color (L*, a*, b*) on raw and cooked patties, proximate analysis on raw patties, and sensory analysis in cooked patties, were conducted after 1, 3 and 5 days of simulated retail display. Highest ($P < 0.05$) lethality was achieved with 1500 psi $_{CP}CO_2$ for 15 min (0.93, 1.00, and 1.06 log reductions for GEC, O157:H7, and SS, respectively). $_{CP}CO_2$ application on beef trimmings showed similar ($P \geq 0.05$) scores for L*, a*, and b*, and proximate values in raw patties. Ground beef patties manufactured from treated beef trimmings scored higher ($P < 0.05$) values for tenderness; similar ($P \geq 0.05$) values for juiciness, beef flavor intensity, or off flavor intensity were recorded. The use of $_{CP}CO_2$ on beef trimmings before grinding can be effective for reducing spoilage and pathogenic microorganisms in beef trimmings with minimal effects on organoleptic characteristics of ground beef.

P2-35 Improved Shelf Life and Microbial Safety of Hams by the Application of Vaporous Biocides

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Introduction: "Vaporex" is a novel, patented technology for surface disinfection of foods. The technique involves application of vaporized biocides such as acetic acid at levels that are not sensorially detectable in finished product.

Purpose: to assess the antibacterial efficacy of the Vaporex process on MAP processed meats.

Methods: Challenge trials were conducted on commercially produced, modified atmosphere-packed ham using acetic acid as the biocide. The trials assessed:

- immediate inactivation by Vaporex treatment of lactic acid bacteria and *L. monocytogenes* inoculated onto the ham
- variability in susceptibility of various species of lactic acid bacteria and strains of *L. monocytogenes* including acid-tolerant strains; and
- long term effect of Vaporex treatment on product shelf life and safety as dictated by the microbial ecology of lactic acid bacteria and *L. monocytogenes* in modified-atmosphere packed ham. Storage trials were conducted at 4°C and 10°C.

Results: The results demonstrated that the Vaporex system can reliably reduce loads of lactic acid bacteria and *L. monocytogenes* on processed meat products, typically by at least two orders of magnitude. Shelf-life extension was demonstrated, with a delay in growth of total viable count at 4°C of 25–30 days, and 8–10 days at 10°C storage, compared to

untreated controls. In addition to initial reductions in *L. monocytogenes* due to Vaporex treatment, growth of *L. monocytogenes* was prevented for at least 46 days at 4°C and at least 29 days at 10°C.

Significance: The Vaporex process is another potential technology available to improve shelf life and safety of processed meats.

P2-36 Control of *Clostridium perfringens* Spores by Green Tea Leaf Extracts during Cooling of Cooked Ground Beef, Chicken and Pork

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Introduction: *Clostridium perfringens* poses a significant risk to the safety of minimally processed meat and poultry products. Green tea is one of the most popular beverages consumed worldwide.

Purpose: The purpose of this study was to investigate the inhibition of *Clostridium perfringens* by two green tea extracts with low (GTL; 141 mg total catechins/g of green tea extract) and high (GTE; 697 mg total catechins/g of extract) catechin levels during abusive chilling of cooked ground beef, chicken, or pork.

Methods: Green tea extracts were mixed into ground beef, chicken or pork at concentrations of 0.5, 1.0 or 2.0% (w/w) along with three-strain spore cocktail to obtain a final concentration of about 3 log spores/g. Meat samples (5 g) were then vacuum-packaged and cooked to 71°C in 1 h in a water bath. Thereafter, meat samples were chilled from 54.5 to 7.2°C in 12, 15, 18 or 21 h and enumerated for *C. perfringens* population by spiral-plating on tryptose-sulfite-cycloserine agar.

Results: Meat cooled from 54.4°C to 7.2°C in 12, 15, 18 or 21 h resulted in significant increases ($P < 0.05$) in germination and outgrowth of *C. perfringens* populations in the ground beef, chicken or pork control samples without GTL or GTE. Supplementation with 0.5 to 2% levels of GTL did not inhibit *C. perfringens* growth from spores. In contrast, addition of 0.5 to 2% levels of GTE to meat resulted in concentration- and time-dependent inhibition of *C. perfringens* growth from spores. At 2% GTE, significant ($P < 0.05$) inhibition of growth occurred at all chill rates for cooked ground beef, chicken or pork.

Significance: These results suggest that widely consumed catechins from green tea can reduce the potential risk of *C. perfringens* spore germination and outgrowth during abusive cooling from 54.4°C to 7.2°C in 12, 15, 18, or 21 h of meat.

P2-37 Combined Efficacy of Lactic Acid, Lauric Arginate and High Hydrostatic Pressure for Inactivating *Listeria monocytogenes* in Vacuum-packaged Cooked Ham

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A study was conducted to evaluate the effectiveness of lactic acid (LA), lauric arginate (LAE), or high hydrostatic pressure (HHP) alone or combined for inactivating *Listeria monocytogenes* in cooked ham. Samples of ham (each 1.0 in x 1.5 in x 0.25 in) were inoculated with a 5-strain mixture of *L. monocytogenes* to give ~ 7.0 log CFU/sample. Inoculated samples were inserted in oxygen barrier bags and 0.25-ml of LA (3% v/v), LAE (5% v/v), or 3% LA + 5% LAE was added to each sample. Ham that received 0.25 ml of distilled water served as control. Samples were vacuum-sealed, pressurized (0, 400, 500, or 600 MPa) at 23°C for 3.0 min, and *L. monocytogenes* survivors were evaluated within 2 h. Samples were pummeled in buffered peptone water (BPW) and serial dilutions (10-fold) of the wash water were plated on modified oxford medium (MOX) agar. Inoculated agar plates were incubated (35 OC) and bacterial colonies were counted at 48 h. In non-pressurized ham, LAE and LA + LAE decreased initial viable numbers of *L. monocytogenes* by 1.75 and 1.66 log, respectively. HHP (440 to 600 MPa) decreased viability of the pathogen in all ham samples ($P < 0.05$). Initial numbers in control decreased by 4.11, 4.71, and 5.02 log, following pressurization at 400, 500, and 600 MPa, respectively. No differences in viable counts were observed among control, LA- and LA + LAE-treated sample pressurized at 400 or 500 MPa ($P > 0.05$). Only LAE-treated ham exhibited the highest log reduction [5.24 log (400 MPa) and 5.71 log (500 MPa)] with numbers of survivors below the detection limit (25 CFU/sample) in 600 MPa-treated ham $P < 0.05$). Based on these results the surface treatment of cooked ham with LAE has good potential for enhancing the inactivation of *L. monocytogenes* by HHP in this popular Ready-to-Eat product.

P2-38 Viability of *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* in Jamaican Jerk Seasoning Paste at 25°C

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Commercial sterilization is not a practical alternative for ensuring the microbial safety of Jamaican jerk seasoning paste due to negative sensory changes. Safety of this oist seasoning depends on its pH, water activity, and antimicrobial activity of added herbs and spices. The purpose of this study was to evaluate the inactivation of four foodborne pathogens in a commercial brand of Jamaican jerk seasoning paste. Samples of seasoning in 10-oz glass jars were inoculated with a 5-serotype mixture of *Salmonella enterica*, or a 5-strain mixture of *Escherichia coli* O157:H7, *Listeria monocytogenes* or *Staphylococcus aureus* to give ~ 6.82 to 7.20 log CFU/ gram. The jars of seasoning were capped and held at 25°C for 28 days. At set time intervals, samples were analyzed for survivors by plating diluted samples on appropriate selective agar media. Inoculated agar plates were incubated at 35°C and bacterial colonies were counted at 48 h. Also, pH and water activity (a_w) of non-inoculated seasoning were measured. Viability of all four pathogens declined rapidly. At 2.0 h viable numbers of *S. enterica*, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* declined by 5.20, 3.48, 5.80, and 4.24 log, respectively ($P < 0.05$). At 4.0 h log reduc-

tions ranged from 4.28 (*S. aureus*) to 7.0 (*S. enterica*). *S. aureus* was most the most resistant pathogen with viable numbers decreasing by 5.60 log at 72 h whereas, the other three pathogens were beyond detection (< 25 CFU/g). None of the four pathogens was detected at 96 h or at subsequent sampling times. Values for pH and a_w ranged from 3.14 to 3.20 and 0.817 to 0.823, respectively. Based on these results, the commercial brand of jerk seasoning paste tested in this study is not a potentially hazardous food as defined by the US Food and Drug Administration Food Code.

P2-39 Recovery of *Listeria monocytogenes* from Various Aqueous Chilling Solutions (Brine)

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Introduction: Brine chilling solutions are used to cool meat products during processing. If a brine chilling solution becomes contaminated with *Listeria monocytogenes* it may result in the transfer of the pathogen to cooked product.

Variables include salt concentration, pH and chlorine levels.

Purpose: USDA-FSIS-OPHS is interested in developing test methods to detect *L. monocytogenes* in brine.

Methods: Preliminary work resulted in a filtering protocol used in combination with chlorine neutralization. Each 500 ml sample was filtered with a glass fiber filter and a 0.45 μ filter to capture *L. monocytogenes* prior to enrichment. Dey Engley Neutralizing Broth was added to samples to neutralize residual chlorine. The filter method was used to test recovery of *L. monocytogenes* from eight brine formulations ranging from 5 to 20% brine, 0 to 5 ppm chlorine and pH 3 to 7. *L. monocytogenes* was recovered and cultured using the method described in USDA-FSIS-Microbiology Laboratory Guidebook, chapter 8. These data were used to decide high and low inoculum levels used in a multi-laboratory study to measure recovery from inoculated and un-inoculated brine samples. Studies were repeated with the addition of the competitors, *Micrococcus luteus* and *Staphylococcus aureus*.

Results: The filtering method along with D/E Neutralizing Broth worked well in conjunction with the standard FSIS protocol for *L. monocytogenes* detection. Limit of detection for *L. monocytogenes* varied by brine formulation. Mild brine formulated at pH 7.5% brine and 0 ppm NaOCl resulted in a limit of detection of 6.9×10^{-3} CFU/ml while a more harsh brine at pH 3, 20% brine and 0 ppm NaOCl resulted in a limit of detection of 7.65×10^{2} CFU/ml. At pH 7, 5% brine, and 5 ppm NaOCl, the limit of detection was less than 1.0×10^{-2} CFU/ml in a 500 ml sample. The addition of competitors appeared to have no effect on the limits of detection.

P2-40 Identification of Microbiological Hazards in the Environment and Processes of Traditional Fermented Sausages

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Introduction: The production of fermented sausages often relies on natural contamination originating from raw materials and processing house-flora or otherwise stated "resident microflora". Therefore, the hygienic status of the processing environment and equipment has an essential role on the microbial stability and safety of these products.

Purpose: The purpose of this study was to investigate the microbial status of small-scale plants producing traditional and/or delicatessen meat products in order to assess the hygienic quality and safety of traditional fermented sausages from the producers to consumers.

Methods: Surface samples were collected from the environment (cold rooms), equipment (tables, stuffing and mincing machines) and utensils (knives) of nine (9) small-scale meat processing workshops. Product samples were taken at different phases (just after stuffing: batter, after fermentation: medium, and final products) of the processing. Microbiological testing was carried out on the swabbed surfaces and product samples. Bacterial numbers were converted to log CFU/cm^2 or log CFU/g and were compared by ANOVA.

Results: Significant variation of microbial counts was evident between different plants as well as between different surfaces ($P < 0.05$). *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococus aureus* were detected in the 18.2%, 31.8% and 9% of the environmental samples respectively. Elevated numbers (> 4-log CFU/cm^2) of *Staphylococcus*/Kocuria, lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas*, yeasts/molds and enterococci were detected in all the environmental samples. Product samples were heavily contaminated with the house-flora present on the surfaces and the equipment of processing plants. The 42.9% of final products were found to be contaminated with *L. monocytogenes*.

Significance: Our findings confirm that inadequate hygiene practices within food-processing plants may result in loss of microbial control; therefore, this study addresses the need for strict control measures within small-scale facilities producing traditional sausages.

P2-41 DSC Survival of *Clostridium perfringens*, *Enterobacteriaceae*, Coliforms and *Escherichia coli* in Tajik Sambusa

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Introduction: Sambusa is an ethnic food of Tajikistan made from ground beef, onions and spices, and baked in triangular shaped dough. Traditionally, sambusas are cooled at room temperatures and

stored without refrigeration after baking. This practice may be hazardous as spore forming pathogens can survive the baking process and grow to hazardous levels during cooling.

Purpose: The purpose of this study was to evaluate the survival of *Clostridium perfringens* spores, aerobic plate counts (APC), *Enterobacteriaceae*, Coliforms and *Escherichia coli* in sambusa during baking and germination and outgrowth of *C. perfringens* spores during cooling at room or refrigerated temperatures.

Methods: Fresh ground beef was inoculated with a three-strain cocktail of *C. perfringens* spores at ca. 3.5 log CFU/g. Sambusas were prepared with 40 g of either inoculated or non-inoculated ground beef mixture, baked for 45 min at 180°C to an internal temperature of 98°C. *C. perfringens* (vegetative cells and spores) populations were enumerated in inoculated products where as APC, *Enterobacteriaceae*, Coliforms and *E. coli* were enumerated in non-inoculated products before and after baking and after cooling at ambient or refrigeration temperatures.

Results: *C. perfringens* spores were able to germinate and grow by 3.18 log CFU/g during cooling at room temperature (21.4°C), whereas the populations decreased by 1.8 log CFU/g during refrigerated storage (5.2°C). APC, *Enterobacteriaceae* and Coliforms populations were reduced by 4.3, 1.96 and 2.55 log CFU/g after baking, respectively. *E. coli* was below detectable levels (0.04 log CFU/g) after baking. *Enterobacteriaceae*, Coliforms and *E. coli* were below detectable levels (0.04 log CFU/g) in the sambusas regardless of cooling at room or refrigeration temperatures.

Significance: It is important to cool sambusa under refrigeration after baking to minimize the risk of foodborne illnesses. These results will assist Tajik small food manufacturing businesses and consumers in understanding proper cooling requirements for sambusa.

P2-42 DSC Fate of *Listeria monocytogenes* on Vacuum-packaged Pepperoni Stored at 4, 12, or 25°C

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Introduction: *Listeria monocytogenes* should be destroyed during processing of pepperoni, but it may be reintroduced during slicing or packaging of the product.

Purpose: In this study we evaluated the fate of *L. monocytogenes* on the surface of inoculated product during storage and potential differences in survival of three types of inocula cultured under different environmental conditions.

Methods: The inocula were composed of 10 strains of *L. monocytogenes* which were cultured separately in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), with or without 1% glucose (acid-adapted and non-habituated inoculum, respectively), or grown in TSBYE (30°C, 24 h) and then habituated in pepperoni extract (10% w/w in distilled water) at 7°C for 72 h (extract-habituated inoculum). Commercially available pepperoni (two replicates, three samples per treatment) was sliced, and inoculated to a level of contamination of 3–4 log CFU/cm^2, before it was vacuum-packaged and stored for up to 180 days at 4, 12, or 25°C. The samples were analyzed periodically for surviving *L. monocytogenes* populations (PALCAM agar) and total bacterial counts (tryptic soy agar with 0.6% yeast extract).

Results: Overall, the levels of the pathogen and total counts decreased continuously during storage, regardless of inoculum type. The pH of the product was relatively stable (4.64–4.82) throughout the storage. When the product was stored at 4°C the levels of acid-adapted inoculum dropped below the detection limit (-0.48 log CFU/cm^2) at 32 days, whereas the other two types of inocula survived for up to 47 days of storage. In product stored at 12°C the non-habituated inoculum survived longer than the other inocula. At 25°C, the extract-habituated inoculum died faster than the other inocula. Thus, the rate of death varied with temperature of storage and type of inoculum.

Significance: The results of this study may be useful in risk assessment studies and in helping processors select proper storage conditions for pepperoni.

P2-43 DSC Survival of *Escherichia coli* O157:H7 on the Surface and Inside Westphalian Ham during Ripening Following Needle Tenderization

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Introduction: Westphalian ham is a dry cured Ready-to-Eat meat product. These artisanal hams are manufactured without a lethal heat treatment and are preserved by salting, fermentation, and drying over ≤ 3 month production period.

Purpose: To determine whether solid needle treatment used to increase the cure penetration rate and accelerate ripening of dry cured hams caused internalization of *E. coli* O157:H7 from external surfaces.

Methods: Prior to needle treatment, hams were immersion-inoculated with a five strain cocktail of *E. coli* O157:H7 while a non-inoculated group served as the control. In two trials replicated four times, *E. coli* O157:H7 were enumerated on sorbitol MacConkey agar containing cefixime and tellurite (CTSMAC), while injured cells were recovered on tryptic soy agar incubated 35°C for 2 h and overlaid with CTSMAC. The ham surface was sampled by removing lean exterior shavings (depth 2–3 mm) while deep tissues were sampled by removing and searing two interior cores (47 mm diameter) from the biceps femoris muscle (which included a minimum of four needle penetrations).

Results: Hams were found to initially contain 7.29 and 4.62 log CFU/g *E. coli* O157:H7 at the surface and internally, respectively. Following 112 days of manufacture including 79 days drying, *E. coli* O157:H7 were not detected on external surfaces after enrichment, whereas in deep tissue at an aw value of 0.88, 3.12 log CFU/g of *E. coli* O157:H7 were recovered.

Significance: The Westphalian ham process used mimicked commercial practice and was not adequate to destroy internalized *E. coli* O157:H7. Needle tenderization was shown to act as a vehicle for internalization of surface bacterial contamination, which allowed greater survival of the pathogen.

P2-44 Inactivation of *Listeria monocytogenes* on the Surface of Cooked Turkey Breast and Roast Beef Using High-pressure Processing and Food Grade Chemicals

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Introduction: *Listeria monocytogenes* is frequently associated with outbreaks and recalls of contaminated Ready-to-Eat red meat and poultry products due to post-processing contamination. Thus, it is necessary to identify treatments that can be applied after processing to reduce the risk of listeriosis.

Purpose: Evaluate the inactivation of *Listeria monocytogenes* on the surface of cooked, uncured turkey breast and cooked roast beef by combining high pressure and food grade chemicals.

Methods: Core samples were aseptically removed from chubs (3 to 5 pounds each) obtained directly from a cooperating manufacturer. The surface of each core sample (4 cm in length and 2 cm in diameter) was uniformly inoculated with a five-strain cocktail (ca. 6.0 to 8.0 log CFU/core) of *L. monocytogenes* and re-packaged in vacuum bags that did or did not contain 2 or 4 ml of antimicrobial. The antimicrobials tested were nisin (Nisaplin at 500 or 1000 ppm) and lauric arginate (LAE at 1 or 10%). The inoculated samples were processed for 5 or 10 min each under 200, 400, or 600 MPa at 20°C.

Results: In general, the greater the time of treatment, the greater the concentration/volume of antimicrobial, and/or the greater the level of pressure applied, the greater the reduction of *L. monocytogenes*. Regardless of the treatment, it was possible to decrease pathogen numbers by at least 1.6 log CFU/core and by as much as 7.4 log CFU/core.

Significance: When used alone, post-processing treatment with HPP or a food grade chemical reduced pathogen levels by 0.2 to 6.1 log CFU/core. HPP in combination with Nisaplin or LAE provided an additional 1.5 log CFU/core reduction. Post-processing treatments with Nisaplin or LAE on cooked, uncured turkey and cooked roast beef followed by pressurization can appreciably improve the safety of these products in case of post-processing contamination.

P2-45 Fate of *Listeria monocytogenes* Inoculated onto the Surface of Soudjouk and Kippered Beef and Stored at Different Temperatures

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Introduction: *Listeria monocytogenes* is the causal agent of listeriosis, a disease that can be serious and is often fatal in susceptible individuals. Listeriosis has typically been associated with consumption of contaminated foods that are capable of supporting growth of *L. monocytogenes* and that are consumed without further cooking. The presence of the pathogen in Ready-to-Eat meat products is not desired and strictly regulated in the United States.

Purpose: Evaluate the viability of *L. monocytogenes* on two Ready-to-Eat (RTE) specialty/ethnic meat products, namely soudjouk and kippered beef, during storage at different temperatures.

Methods: Individual slices (1.5 cm L x 2.0 cm W x 0.8 cm H) of these two products were separately inoculated on both the top and bottom surfaces with a five-strain cocktail of *L. monocytogenes* (6.0 log CFU/slice). Each slice was re-packaged in polyethylene sterile bags that were then vacuum sealed and stored at 4, 10, 21, or 30°C for up 28 days. In each of at least 2 trials, 3 slices were tested on days 0.25, 1, 2, 3, 5, 7, 10, 13, 17, 21, 28 for each storage temperature.

Results: For soudjouk, levels of *L. monocytogenes* decreased by ≥ 5.0 log CFU/slice to below detection (0.7 log CFU/slice) by direct plating after 5, 13, and 26 days when stored at 30, 21, and 10°C, respectively, whereas at 4°C pathogen levels decreased by about 3.0 log CFU/slice after 28 days. For kippered beef, pathogen numbers decreased to below detection within 10 days at 30°C, whereas when the product was stored at 21, 10, or 4°C, pathogen numbers decreased by 5.0, 2.0, and 1.0 log CFU/slice, respectively, within 28 days.

Significance: These data substantiate that kippered beef and soudjouk do not provide a favorable environment for subsequent growth of *L. monocytogenes* that may be present on the surface of these products due to post-process contamination.

P2-46 Fate of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, or *Listeria monocytogenes* on the Surface of Whole Muscle Turkey Jerky

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Introduction: Jerky is a popular shelf-stable snack. Several studies have evaluated effects of beef jerky processes on lethality of foodborne pathogens; however, there is a scarcity of published literature on effects of processes on lethality of poultry jerky.

Purpose: Evaluate the effectiveness of a commercial process that we previously validated for beef jerky towards pathogens during processing of jerky made with whole-muscle turkey breast.

Methods: Approximately 9.5 log CFU/mL of multi-strain mixtures of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, or *Listeria monocytogenes* were separately applied to the surface of strips of turkey breast and treated as follows: (i) non-marinated and inoculated, or ii) inoculated and then marinated (pH 5.4)

in a sealed bag that was tumbled manually and stored at 4°C for 15 min. For each treatment, strips (ca. 15 cm x 5 cm x 5 mm) were separately inoculated with multi-strain mixtures of one of the three pathogens and placed on trays which were positioned on the top, middle, and bottom levels of a loading rack (three strips/pathogen/level). The strips on the rack were loaded into a commercial-scale smokehouse and heated/dried for either 2.5 or 3.5 h at 73.8°C (165°F) or 1.5 or 2.5 h at 82.2°C (180°F) with constant smoking and without addition of humidity.

Results: Heating/drying turkey jerky for either 2.5 or 3.5 h at 165°F or for 1.5 or 2.5 h at 180°F resulted in a >/= 7.0 log CFU/strip reduction of all three pathogens. However, a slightly less reduction (6.6 log CFU/strip) was observed for non-marinated jerky inoculated with *Salmonella* that was heated/dried at 165°F for 2.5 h or 180°F for 1.5 h.

Significance: These data confirm that processing turkey jerky at 3.5 h at 165°F or for 2.5 h at 180°F is adequate for meeting the performance standard of a 7.0-log lethality established for *Salmonella* spp. in Ready-to-Eat poultry.

P2-47 Prevalence of *Salmonella*, *Campylobacter* and *Listeria* on Retail Organic and Kosher Poultry Products

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Introduction: Organic and kosher poultry are raised and processed using specific guidelines in specialized facilities. Compared to conventionally processed poultry, consumers often perceive these alternatively processed products safer and of higher quality.

Purpose: The incidence of three bacterial foodborne pathogens on alternatively processed poultry was determined.

Methods: A total of 353 whole or cut raw poultry samples (104 conventional, 108 kosher, 41 kosher-organic, and 100 organic) from retail stores in Maryland and Virginia were tested for the presence of *Salmonella*, *Campylobacter*, and *Listeria* over an eight-month period. The pathogens were isolated following selective enrichment. Antibiotic susceptibility of *Salmonella* and *Campylobacter* isolates were determined using agar plate disc diffusion and E-Test procedures.

Results: *Salmonella*, *Campylobacter*, and *Listeria* were isolated from 28, 49, and 45% of poultry samples, respectively. *Salmonella* was most frequently isolated from organic poultry samples (40%), as were *Campylobacter* from conventional (69%) and *Listeria* from kosher (67%) poultry. Serovar Kentucky was the most prevalent *Salmonella* serotype among isolates from kosher and organic poultry, while serovars Typhimurium, Enteritidis, and Kentucky were most frequently isolated from conventional poultry. *Salmonella* isolates from organic poultry were more susceptible to the antibiotics tested than those from other sources. While approximately 60% of *Salmonella* isolates from all type of poultry samples were resistant to at least one antibiotic, resistance to at least 6 antibiotics was displayed by 8% and 26% of isolates from organic and non-organic products, respectively. *Campylobacter* isolated from organic poultry were less resistant to ciprofloxacin than those from other samples (10% vs 18%) but a higher percentage of those isolates displayed resistance to tetracycline (73% vs 47%).

Significance: The high incidence of *Salmonella* and *Listeria* contamination associated with alternatively processed poultry samples indicates the needs for continued improvements of rearing and processing technologies to further reduce bacterial contamination of those products.

P2-48 Microbiological Evaluation of Beef Carcasses during Process at Two Slaughter Establishments in Jalisco State, Mexico

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Introduction: At the time of slaughter, meat is essentially a sterile product. However, during the slaughter process, events can occur that contribute to carcass contamination and ultimately, the underlying meat. Any subsequent handling of the meat is also likely to increase the total bacterial contamination on the meat surface and result in cross contamination of freshly exposed surfaces during indirect contact with previously contaminated surfaces.

Purpose: to determine levels of total aerobic (APC), *Enterobacteriaceae* (E), *Escherichia coli* (EC), total coliforms (TCC), and molds (M)/yeasts (Y) on beef carcasses at two mid-sized slaughter establishments in Jalisco State, Mexico.

Methods: A sterile sponge was used to sample a 100 cm^2 area of the rump, flank and brisket regions of 58 and 84 beef carcasses from establishments A and B, respectively, over a period of two months. The samples were collected before and after carcass wash. Each sample was analyzed for APC, E, EC, TCC, and M/Y on 3M Petrifilm™ plates.

Results: Significant differences ($P \leq 0.05$) were found for the populations of microorganisms after evisceration and after carcass wash, and between the two establishments. At establishment A, all counts except molds were lower after washing ($P \leq 0.05$). Mean counts after evisceration and after carcass wash were, APC, 5.2 and 3.7; E 1.8 and 1.1, TTC 1.5 and 0.6; EC 1.2 and 0.5; M 0.9 and 0.9, and Y 1.9 and 1.4 log CFU/cm^2, respectively. For establishment B, the carcass wash resulted in an increase in microbial counts by 0.5 –1.4 log cycles. Mean counts for establishment B before and after carcass wash were, APC 3.8 and 4.3; E 1.1 and 1.7; TCC 0.3 and 1.2; EC -0.07 and 0.8; M 0.3 and 0.7 and Y 0.3 and 0.8, respectively.

Significance: The findings of this study help to identify sources and practices that lead to increased microbial level on carcasses, and highlight the necessity to have SSOP for beef carcass wash at meat establishments.

P2-49 DSC Isolation and Characterization of Thermal Resistance of *Salmonella* spp. from Raw, Frozen Chicken Nuggets, Strips and Pelleted Broiler Feeds

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Introduction: Raw, frozen chicken nuggets/strips are a significant risk factor in contracting foodborne salmonellosis. Salmonellosis from consuming partly cooked chicken nuggets may be due, in part, to *Salmonella* strains with elevated thermal resistances. The pelleting of poultry feed may select for these organisms.

Purpose: To determine the occurrence and characterize *Salmonella* strains contaminating chicken nuggets/strips and pelleted feeds.

Methods: Salmonellae were isolated using Health Canada MFHPB-20 method for the isolation and identification of *Salmonella* from foods. Strains were characterized by serotyping, phage typing, antibiotic resistance typing and by pulsed field gel electrophoresis (PFGE) patterns (ISO 17025) at the National Microbiology Laboratory (Canada). Thermal resistances of *Salmonella* isolates were determined (D- and z- values) using chicken nugget meat heat challenged in a preheated waterbath and recovered on tryptic soya broth supplemented with 15% agar and 0.6% yeast extract with 48 h incubation at 25°C.

Results: Salmonellae were isolated from 25 g samples in 25% (n = 92) of nugget/strip samples, 95% (n = 20) of chicken nugget meat samples and 10% (n = 111) of pelleted feed samples. *S.* Enteritidis with indistinguishable phage type and PFGE pattern was isolated from both pelleted feed and chicken nugget meat in two instances. Data indicated that *S.* Heidelberg strains isolated from chicken nuggets did not have increased thermal resistance (D55 4.54 min, D60 0.36 min, D62 0.11 min and z 1.49°C).

Significance: *S.* Enteritidis isolated from pelleted feed was the same in two instances as in chicken nugget meat.

P2-50 Prevalence of *Campylobacter*, *Enterobacteriaceae*, and Antimicrobial Resistance in Chickens and Guinea Fowls

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Campylobacter and *Salmonella* infections in humans have long been associated with raw poultry. There is concern about antibiotic resistant bacteria or their genetic material being transferred to humans though contaminated poultry. This study was conducted to compare the presence and antimicrobial susceptibility of *Campylobacter* spp., *Salmonella* spp., and other enteric bacteria between chickens and guinea fowls. Birds were reared on enclosed concrete floor housing covered with pine wood shavings litter material. Chickens (n = 20) and guinea fowls (n = 20) carcasses, drinking water (10 ml; n = 20) and litter (10 g; n = 20) were aseptically collected randomly from a poultry farm and analyzed within 1 h of collection. Individual pens served as experimental units and were replicated two times. *Campylobacter* ssp., *Salmonella* spp., and other *Enterobacteriaceae* were isolated and identified using standard selective media and biochemical tests. The isolates were tested for resistance to tetracycline, ampicillin, streptomycin, kanamycin, nalidixic acid, gentamicin, erythomycin, ciprofloxacin, cefoxitin, and colistin using the Kirby Bauer disc diffusion test. *Campylobacter* spp. and *Salmonella* spp. were isolated from 25 and 35%, and 15 and 20% of whole carcass rinses of chickens and guinea fowls, respectively. Although only *Salmonella* spp. were recovered from drinking water, both *Salmonella* spp. and *Campylobacter* spp. were recovered in litter material. *Campylobacter upsaliensis* was recovered in the guinea fowls, while *Klesiella oxytoca* and *Enterobacter sakazakii* were recovered in chickens. *Campylobacter* spp., *Salmonella* spp., and *E. coli* isolates from chickens and guinea fowls were resistance to ampicillin, kanamycin, erythomycin, and nalidixic acid. The results indicate that raw poultry can be potential sources of antibiotic resistant *Campylobacter* spp., *Salmonella* spp., and *E. coli*.

P2-51 Antimicrobial Resistance in *Campylobacter jejuni* and *Campylobacter coli* Isolated from Chicken Carcass Rinsates: Update from the Animal Arm of NARMS

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Introduction: The development of antimicrobial resistance in *Campylobacter* species, particularly *C. jejuni* and *C. coli*, is of public health concern.

Purpose: The purpose of this study was to analyze the minimum inhibitory concentrations (MICs) for antimicrobials used in susceptibility testing of *C. jejuni* and *C. coli* as part of the animal arm of NARMS.

Methods: *Campylobacter* isolates were tested for susceptibility to eight antimicrobials including Azithomycin, Chloramphenicol, Ciprofloxacin, Clindamycin, Erythomycin, Gentamicin, Nalidixic Acid, and Tetracycline. Minimal inhibitory concentrations (MICs) were obtained using E-test (AB Biodisk) from 1998 through 2004. In 2005, a semi-automated broth microdilution system (Trek Diagnostics, Inc.) was used and Florfenicol and Telithomycin, were added. MIC50s and MIC90s were calculated for each year.

Results: From 1998 through 2004, the MIC50s and MIC90s for *C. jejuni* and *C. coli* either remained unchanged or declined. The most dramatic decline was observed for MIC50s to Tetracycline (the 1998 MIC50 was 256 ug/ml for both *C. jejuni* and *C. coli* while in 2005 the MIC50 was 0.50 ug/ml for *C. jejuni* and 0.25 ug/ml for *C. coli*). The MIC50s for 2006 (to date) were within 1–2 dilutions of the 2005 numbers with the exception of Tetracycline which increased to 32 ug/ml for both *C. jejuni* and *C. coli*. Additionally, using a breakpoint of 4 ug/ml for Ciprofloxacin, the

overall percent resistant declined from 15% in 2005 to 8.5% in 2006 for *C. jejuni* and 22.1% in 2005 to 14.7% in 2006 for *C. coli* (preliminary data for 2006). Overall, the percent resistance, regardless of antimicrobial, was higher for *C. coli* compared to *C. jejuni*.

Significance: These data indicate that, in general, the level of resistance, particularly to Ciprofloxacin, among *Campylobacter* isolates is declining. However, resistance to Tetracycline appears to vary and increasing trends in resistance require continued monitoring.

P2-52 Characterization of *Staphylococcus aureus* Strains Isolated from Pig Carcasses

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Introduction: *Staphylococcus aureus* is involved in a variety of infections, and some strains producing staphylococcal enterotoxins (SE) are responsible for foodborne intoxications. In contrast to *S. aureus* isolated from milk and cheese, only few data are available for strains isolated from carcasses and meat.

Purpose: The aim of the study was to identify *S. aureus* out of 337 coagulase positive staphylococci (CPS) previously collected from pig carcasses at two abattoirs (A, B) during slaughter, and to characterize the isolated *S. aureus* to evaluate the epidemiological relationship.

Methods: *S. aureus* were identified by amplification of a specific section of the 16S-23SrRNA intergenic spacer region. *S. aureus* were then examined for egg yolk reaction (Baird-Parker agar), clumping factor/protein A (latex agglutination), antimicrobial susceptibility (Sensititre), and by PCR for mecA and SE genes (sea to sed, seg, sei, sej). After coa gene amplification, strains were typed by coa gene restriction profile (CRP) analysis using HaeIII.

Results: A total of 142 *S. aureus* strains were identified. Among them, 85.2% were egg yolk positive and 98.6% were positive for clumping factor/protein A. Resistances to antimicrobials were found in 36 (25.4%) strains. None of the strains harbored the mecA gene, which is conserved in methicillin-resistant *S. aureus*. SE genes (mainly seg/sei) were detected in 56 strains, and differences between the abattoirs were significant (A: 51.0%, B: 13.6%; $P < 0.05$). CRP analysis resulted in seven patterns, which were commonly found at both abattoirs. During slaughter (abattoir B), four CRP, including the predominating, were identified throughout the process.

Significance: Genotyping revealed a remarkable homogeneity in *S. aureus* from the different abattoirs and process stages. Therefore, the distribution of a limited number of *S. aureus* genotypes may exist in the pig population. The persistence of predominant CRP throughout the slaughter process may be explained by colonization advantages of these types.

P2-53 DSC Meat Packaging Technologies and the Safety of Ground Beef

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Introduction: Recent petitions to FDA and USDA have requested the re-evaluation of CO as an approved packaging component. Research was needed to determine scientific data regarding the safety characteristics of CO and various packaging systems commonly used in the beef industry.

Purpose: The objectives were to determine the safety of ground beef patties packaged with carbon monoxide gas flush, hi-oxygen gas flush, and rosemary oleoresin in modified atmosphere packaging by determining the growth or survival of *E. coli* O157:H7 and *Salmonella* over time.

Methods: Treatments included control (foam tray with film over-wrap), 80% O_2 / 20% CO_2 MAP, MAP with rosemary extract, 0.4% CO, 30% CO_2, 69.6% N2 MAP, 0.4% CO, 30% CO_2, 69.6% N2 MAP with rosemary extract. Samples inoculated with *Salmonella* were plated on XLD agar and *E. coli* O157:H7 on MacConkey agar overlayed with TSA using the thin-layer agar method. Samples were stored in retail cases and were analyzed on days 0, 1, 3, 5, 7, 14, and 21.

Results: After day 7, *E. coli* O157 (5.73 log CFU/g) in the control were significantly higher than in the MAP packages (4.83 to 5.20 log CFU/g). After 21 days, *E. coli* O157 in the control increased to 5.60 log CFU/g which was significantly higher than the MAP packages (3.94 to 4.34 log CFU/g). For *Salmonella* (day 3), samples packed in control had counts of 5.20 log CFU/g while MAP packages were significantly lower (4.50 log CFU/g). After days 14 and 21, *Salmonella* in controls was 4.29 and 4.27 log CFU/g, respectively, which was significantly higher than the MAP packages (4.30 and 3.94 log CFU/g on day 14 and between 3.75 and 4.01 log CFU/g on day 21).

Significance: Modified atmospheres may be inhibitory to foodborne pathogens and may provide added safety to ground beef products.

P2-54 Mechanisms of *Salmonella* Persistence during Chicken Slaughter

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Introduction: *Salmonella* is an important foodborne pathogen known to contaminate chicken meat and which is commonly present throughout chicken processing facilities. It is not clearly understood how and why certain *Salmonella* strains are more persistent and better able to survive during chicken processing.

Purpose: The study was carried out in order to compare 13 *Salmonella* isolates representing strains from both the start and end of 5 chicken processing plants in Australia. Factors which might influence the persistence and survival of *Salmonella* such as their ability to attach to chicken skin, degree of hydrophobicity and their degree of sensitivity to heat and chlorine were then investigated.

Methods: Individual *Salmonella* cell suspensions were inoculated onto chicken thigh skins and strongly attached cells enumerated in a skin attachment assay. Hydrophobicity of the isolates was determined by performing hydrophobic interaction chromatography using two different buffers (PBS buffer pH 7.0 and 4 mol/L sodium chloride in 0.01 mol/L sodium phosphate buffer at pH 7.0). The isolates were exposed to water heated to 60°C for up to 600 s and enumerated at various time points to determine D-values. In a separate experiment strains were exposed to 4 ppm of free-available chlorine at 4°C for 15 min and enumerated.

Results: Hydrophobicity was not found to correlate to the ability of these *Salmonella* isolates to attach to chicken skin. *S.* Sofia isolates however appeared to be significantly more hydrophobic than other serotypes with both buffers ($P < 0.05$). There were no significant differences ($P > 0.05$) between the isolates from the beginning and the end of processing in their ability to survive heat and chlorine stresses.

Significance: *Salmonella* isolates were exposed to the maximum values of heat and chlorine permitted in chicken processing plants but were able to survive. Hydrophobicity is not a factor influencing *Salmonella* attachment to chicken skin.

P2-55 Effect of Acid Adaptation on Thermal Tolerance of *Escherichia coli* O157:H7 and *Salmonella enterica* in Meat Serum

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Escherichia coli O157:H7 and *Salmonella* were adapted to acidic conditions by growing in tryptic soy broth with 1% glucose (TSB + 1%G). Ability of acid adaptation of these pathogens to provide resistance against thermal stress was evaluated in meat serum. Five-strain cocktail of both bacteria were grown separately in TSB and TSB+1%G for 24 h at 37°C to provide cells with or without acid adaptation. Meat serum was prepared from irradiated ground beef and inoculated with a five strain cocktail of either acid adapted or non-adapted *E. coli* O157:H7 and *Salmonella*. Inoculated meat serum was then subjected to heat treatment at 58, 62, and 65°C to determine D-values of the pathogens. Significant differences ($P < 0.05$) were observed in the D-values of acid adapted and non-adapted *E. coli* O157:H7 at 58, 62, and 65°C. Significant difference ($P < 0.05$) in D-values of *Salmonella* was observed at 58 and 62°C, but no statistical difference ($P > 0.05$) was seen at 65°C. D-values were observed to be 22.46 and 10.58 min. at 58°C; 3.58 and 1.38 min at 62°C; 1.02 and 0.75 min at 65°C for acid adapted and non-adapted *E. coli* O157:H7, respectively. D-values of the acid adapted *Salmonella* were 9.36 min at 58°C, 1.66 min at 62°C, and 1.14 min at 65°C whereas the non-adapted counterparts had D-values of 6.44, 0.88, and 0.95 min at 58, 62, and 65°C, respectively. This indicates that acid adaptation of foodborne pathogens provides cross protection against heat treatments.

P2-56 DSC Effect of Freezing, Thawing Method, and Aerobic Storage on the Fate of *Listeria monocytogenes* during Home Storage of Frankfurters

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Introduction: Although surveys have shown that consumers regularly store unopened frankfurter packages in home freezers, little information is available regarding the fate of *L. monocytogenes* during home storage after thawing. When available, instructions recommend thawing in the refrigerator and discourage countertop thawing, while instructions for microwave defrosting are consistently absent.

Purpose: This study examined the effects of antimicrobials, storage time, freezing, thawing method and subsequent home storage on *L. monocytogenes* on frankfurters.

Methods: Packages of inoculated (2 log CFU/cm^2) frankfurters (8 per package, 3 per treatment), formulated without antimicrobials or with 1.5% potassium lactate plus 0.1% sodium diacetate, were stored at 4°C for 6 or 30 days, then frozen (-15°C; 10, 30, or 50 days). Products were thawed under refrigeration (7°C, 24 h), on a countertop (23°C, 8 h), or by microwave defrosting (220 s, 2459 MHz, 1100 watts, followed by 120 s holding). After thawing and during subsequent aerobic storage (7°C; 3, 7, and 14 days), bacterial populations were enumerated on PALCAM agar and tryptic soy agar plus 0.6% yeast extract.

Results: Antimicrobial ingredients completely inhibited ($P < 0.05$) growth of *L. monocytogenes* before freezing and during the entire aerobic storage period (7°C, 14 days) following thawing. For control frankfurters, length of storage before freezing (4°C; 6 or 30 days) resulted in different pathogen levels on unfrozen product (2 or 4 log CFU/cm^2, respectively), while freezing (10, 30, or 50 days) reduced counts by <1.0 log CFU/cm^2. Changes in pathogen counts following thawing were -1.8 to +0.2 log CFU/cm^2, with microwaving resulting in the largest reductions. *L. monocytogenes* populations on control samples, regardless of thawing method, were similar at 7 and 14 days of aerobic storage (3–4 and 5 log CFU/cm^2, respectively). In general, the fate of *L. monocytogenes* during aerobic storage, following thawing, was not influenced by freezing or by thawing method.

Significance: Microwave defrosting should be included in thawing recommendations. Antimicrobials present in frankfurter formulations inhibit growth of *L. monocytogenes*, diminishing the negative impact of extended storage of sealed and/or opened packages.

P2-57 DSC Characterization of Multi-resistant *Salmonella* spp. Isolates Recovered from Commercially Processed Whole Rabbit Carcasses

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The Centers for Disease Control and Prevention estimates that *Salmonella* infections causes an estimated 1.4 million cases of human illnesses and 400 deaths annually in the USA due to foodborne illnesses. The objectives of this study were to determine the antimicrobial resistance, impedimetric and biochemical profiles of *Salmonella* spp. isolated from whole rabbit carcass rinses at four different sampling points (pre-evis, post-evis, pre-chill and post-chill). Two-hundred whole rabbit carcasses selected from four different sampling points were analyzed for *Salmonella* using tetrathionate as an enrichment broth, bismuth sulfite and brilliant green media as selective media. The isolates were characterized for production of sugars, reaction to O and H antisera, and screening using a direct impedmetric assay specific for *Salmonella*. To determine antibiotic resistance profile, 16 different antimicrobial agents (ampicillin, erythomycin, oxacillin, spectinomycin, vancomycin, novobiocin, nitrofurontoin, Rifampin, cephalothin, ciprofloxacin, Gentamicin, trimethoprim, tetracycline, cefixime and cefazolin) were used by disk diffusion method. Seventy-five (37.5%) of rabbit carcasses were positive for *Salmonella* on bismuth sulfite and brilliant green media. A total of 375 isolates were obtained of which 21 (5.6%) were positive for *Salmonella* by direct impedance method. Of the 21 *Salmonella* positive isolates, 17 (4.5%) were positive for *Salmonella* using O and H antisera. Of the 16 antimicrobial agents tested, 69.38% of the isolates from all sampling points were resistant to multiple antimicrobial agents compared to only 25.31% being sensitive to multiple antimicrobial agents. These findings are significant for the rabbit processing industry and to our knowledge, multiple antimicrobial resistance in *Salmonella* recovered from rabbit carcasses have not been reported previously.

P2-58 Comparison of the TEMPO® System, Petrifilm®, and Cultural MPN Procedure for Enumeration of *Escherichia coli*, Coliforms and Total Aerobic Plate Counts from Poultry Samples

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Introduction: Recent innovations in microbiological methods for analysis of food products have been in methods for detection of bacterial pathogens. Petrifilm dehydrated plates are the only significant addition to cultural procedures for indicator organisms in the last 20 years. An automated most probable number (MPN) has been developed utilizing liquid media to enumerate indicator organisms.

Purpose: The purpose of this study was to compare the TEMPO automated procedure with cultural MPN procedures and Petrifilm assays for enumeration of *E. coli*, coliforms, and total aerobic plate counts (TAPC) from chicken samples.

Methods: Post-chill chicken carcasses (n = 360) were rinse sampled in sterile water and ground chicken (n = 250) were stomached in sterile water. The rinsed and stomached samples were serially diluted on the appropriate Petrifilm plate for *E. coli*, coliforms, and TAPC and tested with the bioMérieux TEMPO system, according to instructions.

Results: The correlation between cultural MPN and TEMPO was 0.82 for *E. coli* and 0.81 for coliforms from post-chill chicken rinse samples. When TEMPO was compared to Petrifilm, the correlation for *E. coli*, coliforms, and TAPC was 0.78, 0.58, and 0.90 respectively for chicken rinse samples and 0.54, 0.09, and 0.91 for ground chicken samples. There was good correlation between TEMPO and Petrifilm for TAPC. The lack of correlation with Petrifilm coliform and *E. coli* counts was due to the inability of Petrifilm to distinguish low levels from background microflora.

Significance: The data suggests the TEMPO and Petrifilm procedures give comparable counts for TAPC. The low correlation for *E. coli* and coliforms is explained because of the inability of Petrifilm to enumerate some *E. coli* and coliforms, particularly when there is a high ratio of competing organisms. TEMPO is an automated system requiring little technician time after the initial processing and provides automated reading, calculation of counts and storage of data.

P2-59 Presence of *Campylobacter jejuni* in Poultry Samples of Different Colombian Regions Using Traditional Microbiology and BAX® System

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Introduction: *Campylobacter jejuni* is found in the intestine of domestic animals, particularly poultry, and causes gastroenteritis that may cause Guillian-Barré Syndrome. Studies in Colombia are very scarce due to the lack of its importance as a foodborne disease microorganism, the high costs of the analysis and the absence of regulations and surveillance programs regarding this pathogen. In developing countries the isolation rate of *C. jejuni* ranges from 5–20%.

Purpose: The purpose of this study was to evaluate the presence of *C. jejuni* in poultry samples by the traditional and a molecular method to explore the frequency of this pathogen in four different regions of Colombia.

Methods: A total of 50 samples of poultry viscera and intestinal content were collected from different Departments of Colombia (14-Cundinamarca, 10-Meta, 21-Santander and 5-Cesar). The samples were analyzed simultaneously by traditional microbiology according to the Food and Drug Administration protocol, using Bolton broth and Karmali agar and BAX® (DuPont-Qualicon) system. *C. jejuni* strain ATCC 33291 was used as a positive control.

Results: Considering the 50 samples analyzed, overall frequency of *C. jejuni* was 38%. Regarding the

region, 1 (3.33%) sample from Cundinamarca (3.33%), 1 (10%) from Meta, 16 (76.19%) from Santander and 1 (20%) from Cesar presented *C. jejuni* according to BAX. Nevertheless, only one strain could be recovered by traditional microbiology.

Significance: These data evidence the high frequency of *C. jejuni* in poultry for sale in Colombia, especially in Santander, which is one of the leading departments in poultry production in our country, and thus the need for surveillance of this pathogen. Additionally, the data shows that *C. jejuni* is a fastidious organism that can be falsely absent in food samples when only traditional microbiology is used, but can be easily detected by molecular methods such as BAX system, important for future research and for control of the microorganism and inspection programs.

P2-60 An Automated Immunomagnetic Separation Enzyme-linked Immunoassay for the Detection of *Salmonella* in Poultry Environmental Samples

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An automated visual immunomagnetic separation enzyme linked immunoassay was developed and evaluated for the detection of *Salmonella* in poultry environmental samples. No false positives were observed when challenged with 33 cultures of microorganisms other than Salmonellae, and positive results were obtained with pure cultures of 53 *Salmonella* isolates tested. From post-enrichment *Salmonella* cultures, the limit of detection of the assay was estimated at 10^4 to 10^6 CFU/mL. Application of the assay on 711 naturally contaminated poultry environmental samples achieved a 100% sensitivity and 96.2% specificity. The assay results were compared with those of a culture reference method performed concurrently on the same samples. The assay allows for the identification of positive samples within 1 hour of testing post-enrichment broths.

P2-61 DSC Evaluation of Detection Methods for the Identification of *Listeria* spp. Recovered from Meat Processing Facilities

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To minimize the labor and time associated with conventional culture methods, user-friendly and rapid pathogen detection methods have been developed. We evaluated the specificity and sensitivity of three rapid detection methods [lateral flow immunoprecipitation assay (LFIA), 3M™ Petrifilm™ Environmental *Listeria* Plates (Petrifilm), and polymerase chain reaction (PCR) method] for identification of *Listeria* spp. in environmental samples in comparison to a culture method (ISO 11290-1), traditionally considered the "gold standard." Over a period of eight months, samples were collected from two meat processing facilities in areas handling raw and cooked foods. Samples were collected after sanitation but prior to processing, and during or after processing but prior to sanitation. Facilities were visited until a minimum of 100 positive and 100 negative samples, per detection method, were collected. The culture method identified 110 positive *Listeria* spp. samples, while LFIA, Petrifilm and PCR methods identified 104, 103, and 109 positive samples, respectively. Out of 103 presumptive positive samples identified by the Petrifilm method, only 38% of these samples were confirmed positive by culture methods. LFIA and PCR were highly sensitive and specific when compared to the culture method, with sensitivity values of 94.5% and 99.1%, respectively, and specificity values of 100%. The Petrifilm method was significantly less efficient in detection of *Listeria* spp. with sensitivity and specificity values of 56.3% and 91.5%, respectively. Kappa values indicated excellent agreement of the LFIA and PCR with the culture method, while moderate agreement was observed for the Petrifilm method. Overall, Petrifilm was found to be easy to use but less efficient in detection of *Listeria* spp. in environmental samples, while the LFIA and PCR methods were found to be excellent alternatives to culture, considering performance, time and labor inputs.

P2-62 Evaluation of Sponge-sampling Methods for Fleece and Carcasses of Sheep in a Commercial Abattoir

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Introduction: There is little quantitative information on the numbers of *E. coli*, *E. coli* O157 and *Salmonella* present on sheep fleeces and carcases at processing in Australia. In order to gather information on these pathogens, appropriate sampling methods are required.

Purpose: The purpose of this study was to compare two methods for sampling of sheep fleece and carcases for the enumeration of *E. coli*, *E. coli* O157 and *Salmonella*.

Methods: The fleece and pre-chill carcases of 24 sheep processed at a commercial abattoir in Australia were sampled using a large-area sponging method (LAS; sponging the entire side of the fleece or carcase) and the AQIS ESAM sampling procedure (sponging three 5 cm by 5 cm areas on each of flank, midloin and brisket). *E. coli* counts were determined using Petrifilm™ and the presence of *E. coli* O157 and *Salmonella* was determined using automated immunomagnetic separation and selective plating. *Results*: *E. coli* counts on the fleeces and carcases were significantly higher ($P < 0.001$) with the ESAM method (mean 1.77 log CFU/cm ≤ on fleece, 1.62 log CFU/cm ≤ on carcases) than with the LAS method (mean -0.14 log CFU/cm ≤ on fleece and -0.27 log CFU/cm ≤ on carcases). *Salmonella* counts were higher ($P < 0.001$) on fleece using the ESAM method (mean -0.64 log MPN/cm ≤) than the LAS method (mean -1.97 log MPN/cm ≤). The isolation rate of *Salmonella* from fleece was not significantly different between the ESAM (11/24) and LAS methods (15/24). No comparison could be made for enumeration of *E. coli* O157 on fleece (1 positive sample), or for either pathogen on carcases due to the low isolation rate (1 positive sample for each pathogen).

Significance: The ESAM sampling protocol appears to target heavily contaminated sites and is appropriate for testing fleece and sheep carcases for enumeration of *E. coli*, *E. coli* O157 and *Salmonella* as no advantage was gained in sampling a larger area.

P2-63 The Effect of Incubation Temperature on Total Viable Counts of Beef and Sheep Carcasses

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Monitoring of Total Viable Count (TVC) can provide valuable data for process control in the processing of cattle and sheep for human consumption. *E. coli* data are difficult to use in process control because they are usually below the limit of detection. Australian Standard methods usually employ an incubation for 72 h at a temperature of 30°C (termed, Standard Plate Count), but for meat, the incubation is for a period of 96 h at 25°C (usually termed, Total Viable Count). Petrifilm methods specify incubation at 35°C. An investigation was performed to compare incubation temperatures and method of plating (pour plate/Petrifilm) for determination of TVC. Samples were collected following the sampling methods of the USA Pathogen Reduction; Hazard Analysis Critical Control Point (HACCP) Systems; Final Rule (also known as the "Mega Reg"). Counts incubated at 25 and 30°C on Petrifilm were similar and were 0.5 log and 0.8 log higher (beef and sheep carcasses, respectively) than counts on Petrifilms incubated at 35°C ($P < 0.05$). Counts obtained on pour plates incubated at 25 and 30°C counts were similar but slightly lower than those on Petrifilm at the same temperatures. The work has value in aligning databases both within Australia and internationally where it is noted that in the USA plates are incubated at 35°C and in New Zealand where plates are incubated at 30°C.

P2-64 F-RNA Coliphages as a Potential Model Organism for Enteric Viruses of Animal Origin

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Introduction: There are increasing concerns of zoonotic transmission of some animal enteric viruses which are closely related to human pathogenic strains. Male specific (F+) RNA coliphages, considered a potential model organism for enteric viruses, are part of the mammalian gut flora, are cultivable and have similar survival characteristics but currently there is little information on the contamination of carcasses with F-RNA coliphage and their genotypes.

Purpose: The purpose of this study was to assess the frequency and levels of contamination of hog carcasses with F-RNA coliphages and their genotypes throughout the dressing process.

Methods: Using a direct standard double agar plating method, F-RNA coliphages were enumerated from fecal samples obtained from the large intestine and swab samples obtained from the anal area or random sites of 96 hog carcasses at four points along the dressing line at a research abbatoir. From each positive sample, representative F-RNA plaques were isolated, confirmed, and differentiated into genogroups using a duplex RT-PCR assay.

Results: F-RNA phage were recovered from 43% fecal samples at levels of 10^2–10^5 plaque forming units (PFU)/g and from 35% or 25% of anal or random sites, respectively, before and after evisceration at 10^1–10^3 PFU/100 cm^2. The frequency and recovered numbers of F-RNA coliphage remained similar after carcass pasteurization or washing. For RT-PCR assays of isolates from fecal samples, 66%, 7% or 17% were positive for leviviruses, alloleviviruses, or both, respectively. For 10% or 8% of fecal or random samples, respectively, an RT-PCR product was not obtained. About 69% or 23% of positive random samples contained leviviruses only or both leviviruses and alloleviruses, respectively, while all positive samples from the anal area were leviviruses.

Significance: These findings suggest that larger scale studies of carcass contamination with F-RNA coliphages are warranted to determine their potential as a model organism for enteric viruses.

P2-65 DSC Multiplex Polymerase Chain Reaction Assays for Screening Virulence Genes of *Campylobacter jejuni* Strains Isolated from Processed Broilers

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Introduction: *C. jejuni* is the most important cause of campylobacteriosis, a common intestinal disorder in humans. Campylobacteriosis can be caused by the consumption of uncooked chicken.

Purpose: The purpose of this study was to develop a series of multiplex polymerase chain reaction (PCR) assays to identify the virulence genes of *C. jejuni* strains collected from processed carcasses at different broiler processing facilities. Genes that are associated with colonization and infectivity were chosen for this study.

Methods: DNA was isolated and tested with PCR assays for the presence of flhB, flagellar protein biosynthesis and export gene; flgB, flagellar basal body protein synthesis gene; flgE2, flagellar hook protein gene; docA, colonization and infectivity gene; cdtA, a gene whose product makes the eukaryotic cells to be blocked in either G2 or early M phase. Published primers were used to design the PCR assays.

Results: Thirty-six *C. jejuni* isolates from one processing plant were positive for the presence of flhB, flgB, flgE2 and cdtA genes, but were negative for docA gene. Fifty-eight *C. jejuni* isolates obtained from another poultry processing plant were positive for all these genes, including docA. Multiplex PCR assays were successfully designed for the following gene combinations, flgB, docA and flhB; flgB, flhB and cdtA; and flgB, docA, cdtA. These assays have a similar annealing temperatures.

Significance: This study will result in a series of multiplex PCR assay for rapid identification of virulence genes of *C. jejuni*, and will provide insights into the distribution pattern of these genes in *C. jejuni* strains isolated from processed broilers.

P2-66 Enrichment Media and Protocol Comparison for the Rapid Detection of *Salmonella* in Poultry Carcass Rinses

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Enrichment media and protocol has been demonstrated as an integral part of the rapid detection method for foodborne pathogens. The effect of resuscitation and productivity of the enrichment directly determines the result of rapid detection tests, hence, the method sensitivity and specificity. The recently launched RapidChek® SELECT™ *Salmonella* test kit includes phage supplemented primary media and no-supplement secondary media, and the protocol is a single transfer for the whole method. The primary media showed strong selectivity against growth competitors like *Citrobacter, Klebsiella,* and *E. coli,* and the secondary media produced at least two log higher counts of *Salmonella* than tetrathionate broth, Hajna (TTh) after overnight growth with initial ~10 cells. These media and protocol were compared with reference cultural media, i.e., buffered peptone water, TTh, for *Salmonella* enrichment in poultry carcass rinses, as detected either by cultural streaking method, automated immunoassay, or lateral flow immunoassay. For each method, a total of 627 carcass rinse samples have been tested, including 280 whole bird rinses, 75 spiked rinses and 272 customers' samples. The RapidChek® method reported 130 confirmed positives, with a sensitivity and specificity of 99% each, while the reference method and automated ELISA method reported 116 positives in total. The superior performance of the new test kit, plus its simplicity and ease of use, provides a strong solution for *Salmonella* testing in carcass rinse samples for food safety and HACCP purposes.

P2-67 Incidence of *Campylobacter* from Post-chill Poultry Carcass Rinse Samples by Improved Enrichment Methodologies from a Processing Plant Over a Three-day Sampling Period

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Introduction: Numerous studies have been conducted to improve enrichment procedures to recover injured or stressed *Campylobacter* cells. Recent studies have shown that utilizing TECRA enrichment broth and modifying the standard procedure can increase the number of positive poultry rinse samples.

Purpose: The purpose of this study was to evaluate TECRA and Bolton's enrichment procedures with samples believed to harbor injured, stressed or low levels of cells.

Methods: Post-chill carcass rinses (n = 150) were obtained from a commercial processing facility. Fifty carcasses were pulled from the processing line over two eight-hour processing shifts on each of three consecutive days and rinsed with 100 ml buffered peptone. Several TECRA and Bolton's enrichment procedures where evaluated along with direct plating for recovery of *Campylobacter*. After 7 days kept at 4°C, the positive rinses were re-evaluated by the best TECRA and Bolton's enrichment method to determine whether *Campylobacter* could still be recovered.

Results: Overall, 24/150 samples from direct plating and 86/149 enrichment samples were positive for *Campylobacter*. TECRA broth with delayed addition of antibiotics and reduced temperature for 5 h and incorporation of Campy-gas resulted in the greatest number of positive samples at 74/149. Bolton's procedure with Campy-gas resulted in 46/149 positives. The least effective procedure was Bolton's without gas where only 12/149 samples were positive. The best TECRA and Bolton's enrichment procedures recovered 13 and 2 respectively from 86 confirmed positive rinse samples which were stored at 4°C for 7 days.

Significance: The incorporation of Campy-gas into the procedures and delayed addition of antibiotics in TECRA had the greatest positive effect on recovery of *Campylobacter*. The value of a more sensitive enrichment procedure was best seen after refrigeration stressed where 13 of 86 samples were positive using the best TECRA procedure compared to only 2 of 86 with the best used Bolton's procedure.

P2-68 Efficacy of Several Enrichment Procedures Utilizing TECRA and Bolton's Broth for Recovery of *Campylobacter* from Commercial Poultry Carcass Rinse Samples

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Introduction: Improvements in the cultural recovery methods for *Campylobacter* are essential to accurately assess the epidemiology and ecology of this organism in poultry. In a preliminary study, significantly more *Campylobacter* were recovered from poultry carcasses with TECRA broth than with Bolton's broth.

Purpose: The purpose of this study was to (1) compare recovery of *Campylobacter* from poultry carcass rinse by direct plating and enrichment in TECRA and Bolton's broth and (2) compare delayed addition of antibiotics, incubation temperature, modified atmosphere, and combinations of the three for recovery of *Campylobacter* from post-pick and post-chill poultry carcass rinse samples.

Methods: Post-pick and post-chill carcass rinses (n = 198) were obtained from 20 different processing plants. A matrix was set up to asses five different Bolton's enrichment procedures and eight different TECRA enrichment procedures on recovery of *Campylobacter*. From each carcass rinse samples, an aliquot (5 ml) was placed into 45 ml of enrichment broth (this was performed on all 13 different enrichment procedures). After incubation, an aliquot was streaked from the enrichment onto Campy-Cefex plates and standard laboratory procedures performed. In addition, direct plating including the zero dilution was performed on all carcass rinse samples.

Results: From direct plating, 50/100 post-pick and 4/98 post-chill rinse samples were positive for *Campylobacter*. The delayed addition of antibiotics combined with reduced incubation temperature for 5 h in Campy-gas all contributed to increased recovery of *Campylobacter* from TECRA enrichment broth. The best TECRA enrichment procedure recovered 80/100 post-pick and 38/98 post-chill positive samples. The best Bolton's enrichment procedure recovered 72/100 post-pick and 37/98 post-chill positive samples.

Significance: The utilization of delayed addition of antibiotics with incubation under Campy-gas had the greatest effect on improved recovery of *Campylobacter* cells from rinse samples utilizing TECRA, while delayed addition of antibiotics and reduced temperature had a negative effect on recovery from Bolton's. Direct plating was not a reliable method to determine qualitatively the number of samples positive for *Campylobacter*.

P2-69 An Enumeration Method and Sampling Plan for Mapping the Number and Distribution of *Salmonella* on the Chicken Carcass

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Knowledge of the number and distribution of pathogens on foods will improve food safety by allowing better assessment and management of this important risk to human health. However, knowledge of food pathogen ecology and its response to food production and processing practices is limited. Consequently, a selective plating media with multiple antibiotics (XLH-CATS) and a multiple drug resistant (MDR) strain of *Salmonella* Typhimurium DT104 were used to develop an enumeration method and sampling plan for mapping the number and distribution of *Salmonella* on young chickens in the Cornish game hen class. The enumeration method was based on the concept that the time to detection on XLH-CATS during incubation of chicken parts in buffered peptone water (BPW) is inversely related to the initial log number (No) of *Salmonella*. The sampling plan for mapping involved dividing the chicken into 12 parts that ranged in average size from 42 to 80 g. To develop the enumeration method, the parts were spot inoculated with 0 to 6 log CFU of the MDR strain of DT104 followed by incubation in 300 ml of BPW and detection on XLH-CATS. An inverse relationship between detection time (DT) on XLH-CATS and log No was found: DT (h) = 7.72 – (0.973*N_o); R^2 = 0.968. Type and size of chicken part and brand of chicken did not alter ($P > 0.05$) the linear relationship between DT and N_o. Because DT was variable within N_o among chickens (n = 12), the final linear equation for enumeration included a 95% prediction interval that provided stochastic results for N_o. The enumeration method and sampling plan will be used in future studies to map changes in the number and distribution of *Salmonella* on young chickens fed the MDR strain and subjected to different production and processing practices.

P2-70 Carcass Mapping Study That Investigates Microbial Contamination throughout the Slaughter and Fabrication Process

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Introduction: *Escherichia coli* O157:H7 is the cause of severe bloody diarrhea and hemolytic uremic syndrome (HUS) in humans. Cattle are the primary reservoir of this pathogen and meat is contaminated during slaughter primarily from the hides of the animals. Understanding sources of the contamination is important to control this pathogen.

Purpose: To determine sources of carcass contamination from the hides of incoming cattle and to determine points of cross contamination to or from equipment and the environment.

Methods: A total of three carcasses/hour were tested during the course of the processing day for three days/week during three weeks in a commercial abbatoir. Hides were sampled and carcasses were sampled at pre-evisceration; also, separate carcasses were sampled at the shanks, neck, midline, and round before entering the cooler. All samples were analyzed for the presence of *E. coli* O157:H7.

Results: A total of 229 of 2039 samples collected on the carcasses were positive for *E. coli* O157 (11.23%) and 79 of 127 hide samples were positive (62.2%). All samples were collected during the month of August when prevalence is traditionally high. Of all sampling locations, the inside round samples had the highest number of positives followed by the fore shanks and then the hind shanks. These three sampling locations accounted for more than 60% of all positive carcass samples. Very few positive samples were recovered from the neck or midline.

Significance: An understanding of the distribution of *E. coli* O157 on the carcasses of beef cattle during slaughter can assist the industry in implementing targeted interventions for control of this pathogen.

P2-71 Analysis of Pathogen Control Performance in United States Broiler Slaughter Plants

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Introduction: Salmonella test results provide a measure of pathogen control in meat and poultry produced at slaughter and processing plants. Using data from a USDA/FSIS-sponsored survey of broiler slaughter plant inspections, *Salmonella* test results, and other plant characteristics, potential factors affecting pathogen control were investigated.

Purpose: The purpose of this study was to conduct independent statistical analyses to identify key characteristics of broiler slaughter plants associated with pathogen control as measured by *Salmonella* test results.

Methods: The analysis dataset includes information on 153 federally inspected broiler slaughter plants, of which 111 failed half the performance standard for *Salmonella* at least once from 2003 though 2005. Exploratory data analysis techniques were used to

identify key predictors of pathogen control performance. Emerging predictors were factor analyzed to identify latent determinants of pathogen control performance, the relative importance of which was estimated using logistic regression, with *Salmonella* test results serving as the binary dependent variable.

Results: Plants with a greater percentage of production space over 20 years of age are 3.1 times more likely to fail half the performance standard. All plants with younger production space but that have a sanitation inspection failure rate greater than 5% and that do not use chemical sanitizers failed. Further analyses reveal that latent factors related to pathogen control performance include plant size, level of quality assurance and training, extent of inspection noncompliance, purchases of raw poultry inputs, and age of production space. While plants with higher inspection noncompliance rates and older production space are significantly more likely to fail (odds ratios = 1.4), those that purchase raw poultry inputs are less likely to fail (odds ratio = 0.7).

Significance: Identification of characteristics of broiler slaughter plants that affect pathogen control provides useful information, particularly as it relates to possible merits of a risk-based inspection system.

P2-72 The Effects of Non-intervention HACCP on Microbial Levels on Bovine and Porcine Carcasses at Abattoir

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Introduction: Whether non-intervention Hazard Analysis Critical Control Points (HACCP) is really effective in improving microbial status of carcasses is still being debated. To answer such a question, microbial levels on carcasses before and after HACCP must be compared. This is difficult, because available data on standardized microbiological testing of carcasses, in most countries including within the EU, relate to post-HACCP-implementation only; comparable pre-HACCP data are scarce. In Serbia, such comparisons are still possible, as HACCP implementation in abattoirs started recently.

Purpose: The aim of this study was to compare microbial status of carcasses, determined in the same manner, before and after HACCP implementation so to assess its actual effectiveness.

Methods: Total of 2,880 samples (960 before and 1,920 after HACCP implementation) from dressed carcasses at two slaughter lines (for cattle and pigs) at a single abattoir was investigated. The sampling sites on carcasses (4 per carcass), sampling technique (wet-dry swabs), and microbiological methods for Total Viable Count (TVC) and *Enterobacteriaceae* count (EC) were all as described in EU Commission Decisions 471/2001/EC and 2073/2005/EC. The results were compared using ANOVA (SPSS package).

Results: During a period following the HACCP implementation, average TVC (log CFU/cm^2) on bovine and porcine carcasses decreased (from $P < 0.02$ to $P < 0.001$), compared with a period before the HACCP implementation. The average reductions, depending on the site of the carcass, ranged between 0.9 and 1.5 log/cm^2 on bovine carcasses, and between 0.9 and 2.0 on porcine carcasses. No significant differences in EC were oberved, mainly because a large number of samples did not contain detectable *Enterobacteriaceae*.

Significance: These results suggest that non-intervention HACCP has potential for significant improvement of process hygiene of red meat abattoirs.

P2-73 Regulatory Monitoring for *Campylobacter* in Chickens and on Poultry Meat in New Zealand

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Introduction: Since campylobacteriosis was first made a notifiable disease in New Zealand (NZ) in 1980, the reported annual rate has risen steadily to 370 per 100,000 (2005). In response to this high public health burden, NZFSA has developed a comprehensive risk-based regulatory strategy that involves monitoring of *Campylobacter* levels throughout the food chain, scientific evaluation of a range of control measures, and human epidemiological studies. Scientific work involves development of risk assessment models that are primarily focused on poultry as the predominant source of infection.

Purpose: In order to assess the effectiveness of risk mitigation strategies implemented on-farm and during processing, NZFSA has implemented ongoing national regulatory programs operated under the current National Microbiological Database (NMD) broiler program.

Method: The prevalence of *Campylobacter* in poultry flocks is monitored on a daily basis by sampling, immediately before evisceration, caeca from 10 birds of each cut (pooled for plating to mCCDA) presented to all broiler slaughterhouses. The prevalence and counts of *Campylobacter* on broiler carcasses is monitored by randomly sampling two carcasses each day by each processor immediately after immersion chilling and decontamination wash (if used). Rinse samples are plated to mCCDA.

Results: Data collected to June 2007 and maintained in the NMD databases administered by NZFSA will be presented.

Significance: Monitoring flock prevalence and carcass contamination, with the added advantage of quantification of numbers on carcasses, provides detailed information on *Campylobacter* contamination rates entering and leaving slaughterhouses, and the risk factors associated with high rates. This data will be used to validate risk models and the effectiveness of any on-farm and processing controls that are implemented.

P2-74 Assessment of Cooking Instructions on Labels of Retail, Frozen Ground Beef Patties

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When consumers cook raw, frozen ground beef patties according to the label preparation instructions, they expect the resulting cooked patty to be pathogen-free. This study evaluated the cooking instructions on retail packages of frozen ground beef patties to assess whether the heating times (and temperatures, when given), applied using consumer cook conditions, would produce patties that are heated to the safe internal temperature of 71.1°C (160°F). Cooking instructions on packages of frozen ground beef patties were observed and recorded at 16 retail stores in four western states. Frozen patties were cooked using consumer conditions. Variables included initial temperature of the frozen patty, pre-heating of the pan, cooking temperature, and time/frequency of turnover. The patty internal temperature was measured with five beaded wire, type K thermocouples (TC), located in the center and in each quadrant of the patty, held in a device that inserted the TC tip to one-half the patty thickness. All package labels included Safe Handling Instructions, as required, and most recommended cooking to 160°F. Recommended cooking time, when provided, varied from 1.5 to 9 min per side. The average time required to cook a frozen (-18°C), 112 g patty to 71.1°C (as registered on all five TCs) was 10 min 10 s when the pan was pre-heated to 149°C (medium stove burner setting) and the patty turned once. Starting with a 20°C pan increased average cook time to 11 min 25 s. Starting with a -12°C patty did not affect cook time. A medium-low (107°C) burner setting increased cook time, while a medium-high (204°C) setting decreased it. An average of 12°C difference was observed between the highest and lowest TC; the coldest spot was not always in the middle. Cooking time on labels of some of the frozen ground beef packages was inadequate to heat the frozen patty to 71.1°C throughout. These data suggest that some manufacturers of frozen ground beef patties need to revise the cooking instructions printed on their labels.

P2-75 Food Safety and Inspection Service Strategy to Reduce *Salmonella* Contamination of Broilers

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In 2005 the annual incidence of human salmonellosis in FoodNet sites was 14.5 cases per 100,000 people. This exceeds the Healthy People 2010 objective of 6.8 per 100,000. The FSIS *Salmonella* verification program monitors industry progress toward pathogen reduction. Early in the program, from 1998 though 2000, about 10% of broiler rinse samples tested positive for *Salmonella*, before peaking at 16% in 2005. In February 2006, in response to these increases, FSIS announced changes in its *Salmonella* enforcement strategy, refocusing resources towards establishments with the most positive *Salmonella* tests, particularly those with elevated numbers of serotypes associated with human illness. In addition, FSIS now closely monitors *Salmonella* serotype and pulsed-field-gel-electrophoresis patterns to strengthen attribution of illness to specific products. From June though October 2006, the predominant serotype identified in 630 *Salmonella*-positive broiler samples was Kentucky (52%), a serotype not commonly associated with human illness. Common human-illness serotypes identified included Heidelberg (13%), Enteritidis (12%), Typhimurium (6%), and 4, 5, 12:i (4%). FSIS aims to have 90% of broiler establishments in Category 1 (i.e., six or fewer *Salmonella* positive tests per 51 samples collected on two consecutive sets) and the program will be reviewed in mid-2007 to consider if added measures for enhanced performance are needed. Six months into the program, 45% of broiler establishments were in Category 1, up from 35% at program initiation. These initial results indicate that the program is demonstrating success in aiding the US broiler industry to make progress toward prevention of human salmonellosis.

P2-76 Pathogen Control Strategies Used by United States Meat and Poultry Processing Plants

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Introduction: In 2003, USDA's Food Safety and Inspection Service passed a final rule requiring establishments that produce Ready-to-Eat (RTE) meat and poultry products to perform additional steps in controlling *Listeria monocytogenes*. Processing plants may have implemented pathogen-control practices and other food safety practices in response to the rule.

Purpose: The purpose of this study was to learn about the steps used to control *Listeria* and other pathogens in processed meat and poultry products and other food safety practices.

Methods: We conducted a nationally representative mail survey of processing plants. We received 944 completed surveys (66% response rate). The survey collected information on sanitation practices, use of food safety technologies, and microbiological testing.

Results: Plants employ a variety of sanitation and other food safety practices to control *Listeria* and other pathogens. Most plants sanitize hand tools during operations (89%) and treat drains with sanitizers for pathogen control (84%). About 25% of plants have food safety audits by independent auditors or by their corporate headquarters. About 64% of plants stipulate practices for controlling pathogens in purchase specifications for raw meat and poultry. However, less than one-third of plants apply antimicrobial chemicals or use conveyor belts made of materials designed to prevent bacterial growth. About 70% of plants conduct microbiological testing and environmental sampling. For plants conducting environmental sampling, 84% test for *Listeria* species on a routine basis. In general, large and small plants are more likely to use many types of food safety practices and technologies as compared to very small plants ($P < 0.01$).

Significance: Study findings can be used to establish a baseline of current industry practices and analysis of food safety risk management practices.

P2-77 DSC Educational Needs of United States Cattle Producers Regarding Pre-harvest Food Safety Interventions

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Introduction: Food safety is an important issue facing the beef cattle industry Beef cattle are a reservoir of *E. coli* O157:H7; therefore, food scientists have developed interventions to reduce the prevalence of *E. coli* O157:H7 in live beef cattle prior to slaughter.

Purpose: Interventions are available to cattle producers to aid in reducing the prevalence of *E. coli* O157:H7 at the feedlot. In order to promote the adoption/diffusion processes, it is imperative to understand the knowledge level held by the cattle producers. Once this knowledge level is ascertained, educational materials can be created to target educational gaps.

Methods: Three hundred ninety-one cattle producers were surveyed at the 2006 National Cattleman's Beef Association conference in Denver, Colorado. Questions regarding specific interventions were addressed. Respondents provided feedback on both importance and knowledge of each intervention. Each item was plotted and matrix analysis was used to determine the level of educational need.

Results: Two interventions (vaccines and direct-fed microbials) were determined to be "Successful Programs" while three (neomycin sulfate, sodium chlorate and seaweed/Tasco) were determined to be "Low-Level Needs." No programs were determined to be "Critical Need" from an education standpoint. Demographic information on the cattle producing population was reported. Preferred delivery methods were trade magazines, Internet, and radio broadcasts.

Significance: The successful programs should be monitored to ensure continued success. For all three of the low-level needs, the mean for importance was consistently higher than self-perceived knowledge. While these interventions do not require immediate education materials to be developed, they will require action at a later time. Researchers recommend that materials for pre-harvest food safety be focused on increasing knowledge for these interventions while maintaining educational efforts for the successful programs.

P2-78 DSC Effects of Acid and Cold Stresses on Cell Structure and Growth Kinetics of *Salmonella* Typhimurium in Broth during Storage at 10 and 24°C

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S. Typhimurium cells face mild stresses, such as acid, chilling, and freezing during poultry processing and storage. Resistance to these stresses and ability to repair their injuries during storage may affect the safety of poultry products and human health. The objectives of this study were to investigate growth kinetics of sublethally stressed *S.* Typhimurium in broth at 10 and 24°C and to examine the morphological change of injured and repaired cells using transmission electron microscopy (TEM). *S.* Typhimurium (ATCC 13311) cells were stressed with lactic or acetic acids (0.3, 0.5%) for 10 min, chilled (7°C) and subjected to freezing temperatures (-20°C) for 1 day, respectively. Unstressed, stressed and repaired cells were centrifuged. The collected pellets were fixed with glutaraldehyde for TEM examination. Lag time (LT), specific growth rate (SGR), and maximum population density (MPD) of each growth curve were compared. Exposure to acid and -20°C injured *S.* Typhimurium cells over 90%, while 54% of cells was injured at 7°C chilled temperature. LT and MPD were mainly affected by the method and magnitude of applied stress at both 10 and 24°C. At 10°C, LT of stressed cells by 0.5% acetic acid was extended up to 12 days, while LT of stressed cells by 7°C and -20°C storage temperatures were 3.2 and 6.8 days, respectively. However, SGR was not affected at all by the method of applied stress, except for 0.5% lactic acid, where stressed cells did not repair their injury and died off after 4 days of storage at 10°C. These results indicate that growth kinetics of injured cells was mainly affected by lower storage temperature (10°C) after sublethal stress. On TEM micrographs, most of the stressed cells lost their cellular integrity and membranes partially, whereas both dead and doubling cells were observed after recovery at 30°C for 12 h.

P2-79 Impact of Product Temperature on Quantitative Transfer of *Listeria monocytogenes* during Commercial Slicing of Deli Ham

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Listeria monocytogenes remains more prevalent in deli-sliced than in pre-packaged luncheon meats, with the impact of retail slicing habits on *L. monocytogenes* transfer still not well understood. To assess this problem, a 6-strain *L. monocytogenes* cocktail was inoculated onto the blade of a commercial Hobart delicatessen slicer (Model 2612) by slicing a surface-inoculated ham chub (10^6 CFU/cm^2) to yield ~ 10^3 CFU on the first slice. After tempering ham chubs to 4 and 22°C, a total of 100 consecutive slices was obtained. The first 25 slices were individually diluted 1:5 in UVM and homogenized, after which 5-ml aliquots were pour-plated in duplicate using 25 ml of MOX agar. The remaining UVM-diluted samples were enriched (30°C/24 h) and streaked to MOX for confirmation when *Listeria* was below the detectable limit of 10 CFU/slice. Slices 26–100 were composited into 15 sets of 5 slices, stored at 4°C for 5 days and then enriched for *Listeria*. After slicing, different locations of the slicer were also quantitatively examined for *Listeria* using our 1-ply composite tissue sampling method. Each experiment was replicated 5 times. For the first 25 slices, greater *Listeria* transfer ($P < 0.05$)

occurred when ham was sliced at 22 rather than 4°C. Thereafter, 96% and 98.7% of the composited samples for slices 26 –100 obtained at 4 and 22°C subsequently yielded *Listeria* by enrichment, respectively; *Listeria* was detected in the last composited sample at both slicing temperatures. Although all tested surfaces on the slicer yielded *Listeria* after slicing, the collection table, back plate, and guard back were most heavily contaminated, with greater transfer when slicing product at 22 than 4°C. These findings indicate that product temperature plays an important role in *Listeria* transfer during retail slicing, and that the collection table, guard back, and back plate need to be specifically targeted for cleaning/sanitizing after slicing.

P2-80 Inactivated Autogenous Trivalent *Salmonella* Vaccine Used in Commercial Poultry Breeders for the Control of *Salmonella* Colonization

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Introduction: Colonization of commercial poultry with *Salmonella* can result in human infection through the food chain. The use of single or dual serovar vaccination to control *Salmonella* colonization is well documented, while use of an inactivated trivalent vaccine is unreported.

Purpose: The objective of this study was to develop and use a trivalent vaccine with other intervention strategies to reduce the prevalence of *Salmonella* in commercial breeder flocks and provide maternal protection to the progeny.

Method: An inactivated vaccine was produced using serovars Typhimurium, Mbandaka and Orion, prevalent in commercial poultry in Australia. The efficiency of the trivalent vaccine was first determined using controlled trials that assessed protection of adult hens from colonization by serovars used in the vaccine. Each trial used 50 vaccinated and 50 non-vaccinated 20-week-old commercial Cobb hens. At set intervals, the hens were bled to test for anti-*Salmonella* antibodies and cloacal swabs taken to test for the presence of *Salmonella*. The results were analyzed statistically in a contingency table. The field trials compared the presence of *Salmonella* on an individual shed basis in all poultry breeder farms. Prevalence was determined using drag swab analysis and the serovar profile was plotted over time (months). The field data was analyzed using historical controls.

Results: In the controlled trials, there were significant differences in colonization rate (Fisher exact $P < 0.05$) and anti-Salmonellae antibodies (Student t-test $P < 0.05$) between vaccinated and non-vaccinated birds. The field trial data revealed a decrease in prevalence from 50% prior to vaccination to 40% post-vaccination. Flock age-based analysis demonstrated a decrease in prevalence in all groups compared to pre-vaccination. A change in the profile of salmonellae isolated from breeder flocks was observed after vaccination.

Significance: Based on this study, the trivalent vaccine has been incorporated into an integrated *Salmonella* control strategy.

P3-01 A Decline in the Proportion of Foodborne Outbreaks with Undetermined Etiology Following Increased Specimen Collection and More Rapid Investigation: FoodNet 2001–2005

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Introduction: Outbreak investigations are vital for understanding the epidemiology of foodborne disease and identifying previously unrecognized foodborne pathogens. Adequate specimen collection and prompt investigation are important factors for determining an etiology during an outbreak investigation.

Methods: We analyzed data from CDC's national electronic Foodborne Outbreak Reporting System (eFORS) and supplemental outbreak forms completed by states participating in the Foodborne Diseases Active Surveillance Network (FoodNet) from 2001–2005 to assess the barriers to determining an outbreak etiology.

Results: There were 518 outbreaks reported to eFORS 2001–2002, 83% with a completed supplemental form. There were 689 outbreaks reported from 2003–2005; 85% with a supplemental form. The proportion of outbreaks with an undetermined etiology decreased from 21% in 2001–2002 to 12% in 2003–2005 ($P < .001$). During these two time periods, the percentage of outbreaks with >2 human stool specimens screened for bacterial pathogens or norovirus increased from 56% to 76% (P value <.001), and the mean number of specimens submitted per outbreak increased from 7.5 to 12 ($P < .05$). The proportion of outbreaks investigated within one week of first illness onset increased from 44% to 62% ($P < .001$) between the two time periods.

Conclusion: Between 2001–2002 and 2003–2005, an increase in the number of stool specimens collected, and a decrease in the time lag between illness onset and initiation of health department investigation, was accompanied by a corresponding decrease in the number of outbreaks of undetermined etiology. These data demonstrate that specimen collection and prompt investigation are important factors for determining an etiology during an outbreak investigation and should be encouraged.

P3-02 Outbreaks by the Numbers: Viruses

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Viruses account for 23% (n = 1138/5000) of reported foodborne illness outbreaks with an identified food source from 1990 to 2004, according to data from the Center for Science in the Public Interest. This compares with 61% (n = 3065) linked to pathogenic bacteria, 15% (n = 733) linked to chemi-

cals, and 1% linked to parasites (n = 63). Due to better data collection and improved laboratory detection, viruses have been growing as a reported cause of outbreaks. For example, in the years 1990–1997, viruses were linked to 7.3% (n = 110/1515) of the foodborne illness outbreaks with known or suspected pathogen and identified food. Between 1998 and 2004, they constituted 29.5% of such outbreaks (n = 1029/3485). Norovirus alone is responsible for 88% (n = 1002/1138) of virus outbreaks and 20% (n = 1002/5000) of all foodborne illness outbreaks. Hepatitis A and unspecified viruses account for the remainder of virus outbreaks. Most virus outbreaks occur in restaurants (44%, n = 508) and private homes (13%, n = 144). Most virus outbreaks involve multi-ingredient foods or produce. Multi-ingredient foods, such as sandwiches and salads, are involved in 31% (n = 356) of all virus outbreaks. Produce items, such as leafy green salads, lettuce, and fruit, are involved in 26% (n = 296) of virus outbreaks. The average size of a virus outbreak is 38 cases. Virus outbreaks are underreported for many reasons. Due to the short duration of illness for common foodborne viruses, such as norovirus, ill patients may not visit the doctor. Furthermore, norovirus is not a reportable disease, so doctors have no incentive to report cases to health departments or CDC. Viruses are not usually captured as part of FoodNet or Pulse-Net, so outbreak data with an attributed food source remains the best single source of information on the public health impact of viruses.

P3-03 Outbreaks by the Numbers: Fruits and Vegetables

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The year 2006 was a banner year for produce-related foodborne illness outbreaks, marked by outbreaks linked to spinach, tomatoes and lettuce. In a comprehensive survey of outbreaks with an identified food source, produce accounted for 13% (639/5000) of outbreaks and 21% (31,496/152,097) of illnesses from 1990 though 2004, according to data from the Center for Science in the Public Interest (CSPI). CSPI conducted a hazard analysis on produce by identifying the most common food/pathogen combinations and ranked their risk based on their history of causing outbreaks and associated illnesses: Salad contaminated with norovirus was the most common cause of outbreaks (128 outbreaks/4720 cases), followed by sprouts with *Salmonella* (24/1875), lettuce with norovirus (24/835), salads with *Salmonella* (17/941), melon with *Salmonella* (16/1137), salad with *E. coli* O157:H7 (14/782), tomatoes with *Salmonella* (11/1652), berries with the parasite *Cyclospora* (10/2758), scallions with hepatitis A virus (7/1070), and tomatoes with *Shigella* (1/886). In all produce outbreaks, norovirus is the top cause of outbreaks (39.3%, 251/639), followed by *Salmonella* (19%, 120/639), *E. coli* (8%, 48/639) and *Clostridium* (7%, 42/639). The main hazards associated with salad and lettuce outbreaks are Norovirus (60%, 152/255), *E. coli* (21%, 26/255), and *Salmonella* (19%, 24/255). In light of the recent produce-related outbreaks, identifying ways to control hazards will reduce the risk of foodborne illness from produce. By identifying food/pathogen combinations responsible for produce outbreaks, we can generate a hazard analysis, which is the first step in identifying appropriate solutions.

P3-04 FDA's Use of Epidemiologic Data, Traceback Investigations and Farm Investigations as Regulatory Tools during Outbreaks of *Cyclospora cayetanensis* Associated with Produce, 1995–2005

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Introduction: *Cyclospora cayetanensis* became recognized as a foodborne pathogen for the first time in the US after several outbreaks of foodborne illness in 1996. In 1997, the federal government created the Food Safety Initiative (FSI) to improve food safety and help reduce the incidence of foodborne illnesses, particularly those associated with produce. FDA, in collaboration with other government partners, utilizes outbreak information from epidemiologic, traceback and farm investigations to determine the food vehicle, source, distribution and production chain of the implicated products.

Purpose: The purpose of this study is to discuss the utilization of epidemiologic data and information from traceback and farm investigations by FDA during *Cyclospora* outbreaks from 1995–2005.

Methods: We conducted a PubMed search for published cyclosporiasis outbreaks associated with produce in the US from 1995–2005. We also conducted an electronic search of the FDA's Center for Food Safety and Applied Nutrition's Food Emergency Response (FER) outbreak folders providing a listing of produce-related *Cyclospora* outbreaks. We reviewed the published literature and FER documents to extract pertinent information regarding epidemiological data, and from traceback and farm investigations.

Results: We identified 19 outbreaks of produce-associated cyclosporiasis since 1995. Epidemiologic data revealed 10 outbreaks due to consumption of raspberries, 6 from basil, 2 from mesclun lettuce and 1 from snow peas, causing approximately 3,857 illnesses. Seventeen traceback and multiple farm investigations helped identify specific domestic and foreign farms as the possible source of the contaminated products.

Significance: FDA utilizes epidemiologic data, traceback investigations and farm investigations as tools to support actions, such as product recalls and press releases.

P3-05 Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease

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All foodborne illness is fundamentally preventable, and by influencing human behavior, human illness can be decreased. It has been estimated that approximately 20% of foodborne illnesses of bacterial origin result from transmission by an infected food handler. A review of 816 outbreaks between 1927 and 2006 was conducted by the International Association for Food Protection to develop a clear understanding of the dynamics of pathogen transmission via the food

handler in a variety of settings. Outbreaks included those in homes, restaurants, institutions, processing plants, and farms, both in the United States and in other countries. There were 80,682 cases reported; of these, 2,740 (3.4%) required hospitalization and 98 (0.12%) died. In general, salads (potato, egg, turkey, tofu, and tuna) contaminated with norovirus, *Salmonella*, *Shigella* or *Streptococcus* caused the largest number of outbreaks. Outbreaks were sorted into different categories based on: how many workers were implicated; the origin of the infective agent (whether in the outbreak setting or offsite); the degree of certainty that the worker(s) were the cause or were victims; whether or not the workers denied illness; the ability of the agent to grow in the food; whether only the workers and not the patrons were ill; and whether patrons, rather than the workers, were responsible for their illnesses. The most frequent scenario (238 outbreaks) involved a single food worker as the sole cause of disease transmission, but there were several more complex situations. Contamination of food by workers occurred though cross contamination (usually a raw food of animal origin), the fecal-oral route, by nasopharyngeal excretions or skin lesions. Although workers may be associated with an outbreak, often a clear initiating source is not identified, or workers may be victims as well as sources of infection. The different routes of infection portray a complex picture requiring analysis before implementing effective controls.

P3-06 Prevalence of Foodborne Pathogens in Stools from Mexican Hospitals

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Introduction: Feces are an important source of contamination of foods. Intrahospital infections occur frequently as a result of consumption of contaminated food. Thus, it could be important to analyze the feces of hospital patients to determine the presence of foodborne pathogens that could be a hazard for other susceptible patients.

Purpose: The purpose of this study was to evaluate the presence of several foodborne pathogens in human stools that could be a source of contamination of foods.

Methods: One-hundred samples of human stools were collected from October 2005 to July 2006 from local hospital patients in Monterrey N.L. Mexico and the metropolitan area. Samples were examined for the presence of several foodborne pathogens, including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia coli* O157:H7, *Listeria* spp. and *Yersinia* spp. The presence of pathogens was determined using the Mexican Official Methods and the Bacteriological Analytical Manual (BAM) protocols.

Results: *E. coli* O157:H7 was isolated in 5% of the samples, *Salmonella* spp. in 4% and *Campylobacter* spp. in 1%. *Shigella* spp., *Listeria* spp. and *Yersinia* spp. were not detected in any of the human stools.

Significance: The presence of highly pathogenic foodborne bacteria in feces of hospitalized patients could present a source of infection for other susceptible patients and for contamination of foods.

P3-07 A Novel Approach for Conducting Environmental Investigations of Foodborne Outbreaks

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Environmental investigations of foodborne outbreaks are often complex and take several weeks or months to complete thoroughly. Investigations involve completing epidemiologic studies of ill individuals, tracing products from table-to-farm, seeking contamination sources/modes, testing numerous samples, and writing detailed reports. Because of scope and complexity of these environmental investigations and the fact that contamination events likely happened weeks/months before, public health agencies face challenges in determining exact source(s) and contamination methods, in avoiding duplication of effort and implementing targeted preventive measures to decrease recurrence risk. To increase efficiency, communication, and effectiveness of investigations, the California Department of Health Services in partnership with US Food and Drug Administration's San Francisco and Los Angeles District Offices formed the California Food Emergency Response Team (CalFERT) in 2005. CalFERT's focus is developing a team of highly trained investigators, microbiologists, chemists, and epidemiologists from multiple federal and state agencies to collaboratively investigate foodborne outbreaks expeditiously and effectively. Members meet regularly to receive training on investigation protocols and sampling techniques at processor, retailer, and farm levels. Exercises are conducted to continually refine existing procedures and develop new techniques. During the *Escherichia coli* O157:H7 outbreaks in fall and winter of 2006, teams of investigators from four different federal and state agencies conducted investigations of dozens of possible farms and processors and collected >1,000 environmental samples. Of these samples, several genetically matched the *E. coli* O157:H7 outbreak strains. This enabled investigators to narrow the focus of investigations and gain valuable clues as to source. Pooling limited resources allows agencies to efficiently investigate numerous leads, increase sampling size, increase opportunities to find clues to contamination source(s), reduce redundancy, improve efficiency and effectiveness of investigations, and provide opportunities for investigators to meet and train together to develop trust, expertise and shared experiences. Together, this creates a highly specialized and trained investigation team.

P3-08 DSC Response of *Salmonella* Strains to All Natural Citrus Antimicrobials

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Introduction: The Healthy People 2010 Initiative established a goal of no more than 6.8 cases of salmonellosis/100,000 persons, 1/2 of the initial

baseline rate of 13.6 cases of salmonellosis/100,000 persons. Unfortunately, the most recent data for salmonellosis show a rate of infection of 16.2 cases per 100,000. *Salmonella* mediated foodborne illness continues to be a serious problem. There are many foodborne vehicles, but contaminated poultry meat has been implicated as a major contributor to salmonellosis.

Purpose: The purpose of this study was to evaluate all natural citrus compounds as antimicrobials with action against *Salmonella* spp. of poultry origin.

Methods: Several strains of *Salmonella* were grown in nutrient broth overnight; an aliquot was streaked on BHIA and allowed to dry. Sterile filter paper disks were placed on the plates and 0.01 ml of dilutions of citrus essential oils was aseptically transferred to the disk. Plates were incubated at 37°C for 24 h and zones of inhibition were measured and compared to controls.

Results: FL Valencia whole oil and combined orange terpenes had little to no effect on strains tested. Valencia 5-fold concentrated oil was highly active against S. Typhimurium ATCC 13311, producing a 36-mm zone of inhibition as compared to 6 mm for the control. The terpene hydrocarbon fraction, composed principally of d-limonene, was found to have high activity against S. Typhimurium ATCC 14028 (23 mm), S. Typhimurium ATCC 13311 (25 mm) and a USDA S. Typhimurium isolate (20 mm).

Significance: Citrus-derived antimicrobials are ideal candidates for use in the poultry industry. There is a wide range of commercially available compounds and they offer the potential for development of multiple antimicrobial intervention strategies, so "tailored compounds" can be formulated to minimize specific pathogens as part of multiple intervention steps.

P3-09 *Vibrio parahaemolyticus* Illnesses in Florida, 1994–2006

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The *Vibrio* species of bacteria occur naturally in the marine and estuarine waters of Florida unlike other bacterial pathogens of public health concern, which are introduced to Florida waters by fecal contamination. As expected, seafood consumption is consistently associated with *Vibrio* infection. Oysters, blue crabs, clams, shrimp and fish will harbor the *Vibrio* bacteria naturally and without ill effect to themselves. *Vibrio parahaemolyticus* is one of three *Vibrio* species of primary public health concern in Florida. Infection by this pathogen is acquired by consumption of seafood, exposure to contaminated water and directly though wounds. This illness is characterized by abdominal cramps and watery dysenteric diarrhea. Systemic infection and death is rare. *Vibrio parahaemolyticus* infection may occur as single cases or in large outbreaks. Oyster consumption has been implicated in Florida and multi-state outbreaks. Smaller outbreaks are caused by consumption of cross-contaminated cooked blue crabs. From 1995–2006 there have been 40 confirmed and suspected outbreaks of *Vibrio parahaemolyticus* in Florida. Six confirmed outbreaks were associated with consumption of blue crab; raw oyster consumption was associated with three confirmed outbreaks. In Florida, however, most reported *Vibrio parahaemolyticus* cases are sporadic and are not outbreak related. Blue crab and oysters are both naturally exposed to and carry *Vibrio* organisms that can cause illness upon consumption. However, outbreaks associated with blue crab consumption is a result of poor food-handling practices, post-cooking cross contamination and inadequate temperature control, rather than just consumption of raw or undercooked oysters.

P3-10 Foodborne Sources of Uropathogenic *Escherichia coli*

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Introduction: Urinary tract bacterial disease is a major health problem predominantly caused by *Escherichia coli* strains referred to as uropathogenic *E. coli* (UPEC). UPEC sources are still unclear, but studies suggest a foodborne transmission route. Several virulence gene markers have been identified in clinical UPEC isolates. UPEC antimicrobial resistance (AMR) is also an important problem, with patient management being complicated by multi-drug resistant *E. coli* often carrying AMR integrons.

Purpose: The purpose of this study was to investigate retail meat for the presence of *E. coli* strains carrying uropathogenic marker and AMR genes.

Methods: Modified *E. coli* enrichment broth cultures from 160 beef, pork, lamb and chicken samples collected from retail meat outlets were screened for 7 UPEC-associated virulence genes using PCR. *E. coli* isolates with UPEC-associated virulence genes were isolated using the hydrophobic grid membrane filtration technique and confirmatory PCR. Potential UPEC isolates were then tested for antimicrobial susceptibility, the presence of class one integrons, and isolate relatedness by use of pulsed field gel electrophoresis.

Results: Thirty-six *E. coli* strains containing a number of different virulence factors, including: *papG* (Classes II and III), *hly*, *cnf1*, *ibeA* and *usp*, were isolated. Most isolates originated from chicken (86%), with fewer recovered from beef (11%) and pork (3%). A high prevalence of trimethoprim (75%), trimethoprim-sulfameth-oxazole (70%) and ampicillin (42%) resistance was observed. Twenty-three of 36 (64%) isolates contained class 1 integrons, 17 of which contained *dfr* gene cassettes encoding trimethoprim resistance. Most of the 36 isolates were clonally unrelated but formed 4 clusters at 50% similarity.

Significance: Chicken, and to a lesser extent beef and pork, may be sources of *E. coli* carrying UPEC-associated virulence markers and AMR resistance genes. The potential of retail meat to disseminate antimicrobial resistant *E. coli* UPEC should be further investigated.

P3-11 Characterization and Virulence of *Enterobacter sakazakii* from a Neonatal Intensive Care Unit Outbreak

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Enterobacter sakazakii is a ubiquitous bacterium that has been associated with neonatal infections. Despite considerable recent publicity about the organism, there are relatively few reported outbreaks, and very little is known concerning virulence determinants. This study concerns an outbreak of *Enterobacter sakazakii* which involved 16 neonates. It occurred in a neonatal intensive care unit and resulted in three deaths. All neonates, except for one, were pre-term. Nine neonates had severe clinical symptoms; 7 had necrotizing enterocolitis (NEC) of 1 grade IIIb with abdominal perforation, one with septicaemia, one with meningitis. An autopsy of the latter neonate revealed cerebral lesions. Four neonates were colonized by *E. sakazakii* without any clinical signs, and 2 neonates had moderate digestive problems. A total of 38 *E. sakazakii* strains were isolated from various anatomical sites and 7 strains from powdered infant formula and reconstituted feed. The identity of the isolates was confirmed as *E. sakazakii* using 16S rDNA sequence analysis. Strains were genotyped using Pulsed Field Gel Electrophoresis (PFGE). Antibiotic resistance and plasmid profiles were determined. The virulence of selected strains was compared using attachment and invasion assays of CaCo-2 cells, macrophage uptake, and invasion of rat brain capillary endothelial cells. All strains were in 16S rDNA cluster group 1. PFGE analysis showed that there were three overlapping outbreaks. A fourth (non-matching) genotype was isolated from powdered infant formula. More than one genotype was recovered from one neonate. A number of neonates were colonized by *E. sakazakii* and were asymptomatic. Genotype 2 was associated with the majority of NEC and the meningitis. Strains within a genotype varied in their plasmid profiles. *E. sakazakii* strains were shown to attach and invade CaCo-2 (epithlial) cells, rat brain endothlial cells, and macrophage cells.

P3-12 DSC Food Poisoning Outbreaks Caused by *Bacillus* spp. in British Columbia, Canada

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Introduction: *Bacillus cereus* are environmentally ubiquitous soil organisms with the potential to cause food poisoning. The *Bacillus cereus* complex is highly related phylogenetically, making differentiation between species difficult.

Purpose: 204 archived strains of *Bacillus* spp. associated with food poisoning between 1991 and 2005 were speciated to determine if *B. thuringiensis* had been misidentified as *B. cereus*. *Bacillus* found on imported strawberries between January and March 2006 was also tested.

Methods: PCR was used to detect pathogenicity genes for emetic cereulide toxin (NRPS, non-ribosomal peptide synthetase), insecticidal crystal protein (ICP, cry), and entertoxins (non-haemolytic enterotoxin; *nhe* and hemolysin BL, *hbl*). Strains positive for NRPS were designated as *Bacillus cereus*, those positive for ICP by microscopy or PCR as *B. thuringiensis*. Rhizoidal growth on nutrient agar were identified as *B. mycoides*, and all other strains with typical *B. cereus* phenotype as the *Bacillus cereus* group. Two commercial enterotoxin kits were compared to *nhe* and *hbl*.

Results: 168 (82%) isolates were associated with 40 *Bacillus* food poisoning outbreaks. *B. cereus* was found in 5 (12.5%) outbreaks, *B. thuringiensis* in 4 (10%), *B. cereus* group in 18 (45%) and *B. mycoides* in 1 (2.5%) of the outbreaks. *B. thuringiensis* was detected in another 4 of 12 mixed *Bacillus* spp. outbreaks. These outbreaks caused 202 illnesses (AR, 32%), with an average incubation period of 5.9 h and duration of 24 h. Symptom profiles were significantly different between *Bacillus* species. *B. thuringiensis* was identified in 95% (177 of 187) isolates from imported strawberries. No difference in *Bacillus* levels was detected between countries of origin but higher levels were detected with specific producers. Twenty-eight *nhe* and *hbl* enterotoxigenic patterns were found and the presence of enterotoxin correlated with only one of the commercial kits. ICP detection by *cry* PCR was more sensitive (99.453%) than light microscopy (90.961%).

Signficance: Routine identification of *Bacillus* isolates can be facilitated using molecular methods to detect NRPS and *cry* genes. This study demonstrates that *B. thuringiensis* should be considered a foodborne pathogen.

P3-13 Estimation of the Burden of Gastroenteric Diseases in Miyagi Prefecture, Japan, Using Physician Consultation Rates from a Retrospective Cross-sectional Telephone Survey

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Introduction: At IAFP 2006, we reported preliminary results on the burden of gastroenteric diseases in Miyagi Prefecture, Japan, in which the physician consultation rates were estimated by analyzing foodborne outbreak investigation data for each pathogen. Based on expert opinions, our previous physician consultation rates seemed to be overestimated, considering the bias associated with outbreak circumstances.

Purpose: The purpose of this study was to estimate the burden of illness associated with *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus*, and to improve accuracy in estimating the physician consultation rates though a population based telephone survey in Miyagi Prefecture.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories in Miyagi Prefecture, in the northern part of Japan (population: 2.36 million), from April 2005 to March 2006. The stool sampling rate was estimated using physician questionnaire survey conducted in April 2006. The physician consultation rates were estimated from a retrospective cross-sectional telephone survey conducted in the same prefecture. The questionnaire in the survey was designed to be comparable with the United States, Irish, Canadian and Malta studies. Each factor was multiplied by the laboratory-confirmed cases.

Results: The estimated physician consultation rate was 38.6%. This is approximately 60% of the previously used physician consultation rate estimated from the outbreak investigation data. Consequently, the estimated number of illness per 100 thousand in this region was 142 for *Campylobacter*, 19 for *Salmonella* and 8 for *Vibrio parahaemolyticus*.

Significance: This new data supports our previous study, and reveals that a significant difference exists between our estimate of burden of illness and the reported cases. The uncertainty associated with physician consultation rate was reduced, and the estimation of the burden of gastroenteric diseases needs to be more accurate. However, there are still uncertainty factors that need to be reduced, such as stool sampling rates by physicians.

P3-14 Molecular Characterization, Serotyping and Antimicrobial Resistance Profiles of *Salmonella* Isolates Obtained from Wastewater, Food and Human Sources in Guadalajara, Mexico

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Introduction: Systematic knowledge of the prevalence of serotypes of *Salmonella* and PFGE types and antimicrobial resistance profiles is a helpful tool in *Salmonella* surveillance and provides information about ecology of pathogens, contributing to an improvement in the surveillance of foodborne salmonellosis.

Purpose: The purpose of this study was to identify the serotypes, PFGE patterns and antimicrobial resistance profiles in *Salmonella* isolates obtained from human, food and wastewater sources in Guadalajara, Mexico.

Methods: Between January 2004 and September 2005, 238 isolates were obtained from wastewater (189), food (17) and human (32) samples.

Results: The serotypes isolated from wastewater were Sinstorf (23), Agona (3), Anatum (14), and Infantis (11). Serotypes isolated from foods were Anatum (4) Agona (3), Saintpaul (3) and Typhimurium (3), and from human feces were Typhimurium (8), Enteritidis (6), Agona (3) and Infantis (3). Resistance to multiple antimicrobials was observed among isolates from all 3 sources. Multi resistant *Salmonella* Typhimurium strains were isolated from foods (2), wastewater (1), and humans. All these isolates were resistant to ampicillin, amoxicillin + clavulanic acid, nalidixic acid, chloramphenicol and gentamicin, and intermediately resistant to cefotaxime, ceftazidime and ceftriaxone. One *S.* Newport strain isolated from a beef sample was resistant to ampicillin, amoxicillin + clavulanic acid and chloramphenicol, with intermediate resistance to cephalosporines. PFGE patterns were obtained from 29 isolates corresponding to serotypes Infantis, Anatum, Thompson, London, Muenster and Sinstorf. The same PFE type was found in isolates of *S.* Infantis coming from 2 human samples and 1 wastewater. For serotypes Sinstorf, Thompson London and Muenster from wastewater, the same PFGE type was found during samplings at different times.

Significance: This suggests the prevalence of these clonal lineages in the *Salmonella* populations in Guadalajara. The above data can be useful in *Salmonella* surveillance in Mexico.

P3-15 Trends in Foodborne Outbreaks Caused by School Lunches and Evaluation of Activities to Improve School Lunch Sanitation in Japan

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Introduction: Since outbreaks due to school lunches contaminated with *E. coli* O157:H7 in 1996, Japanese schools have improved school lunch sanitation systems. The number of outbreaks caused by school lunches has dramatically decreased, but new problems have emerged. Critical review and analysis of the hygiene systems and recent outbreaks related to school lunches are needed.

Purpose: The purpose of this study was to characterize trends in foodborne outbreaks caused by school lunch in Japan, to analyze source of contamination and causative practices, and to evaluate the effects of inspecting and training.

Methods: Foodborne outbreak statistics from fiscal year 1996 to 2005 collected by the Ministry of Education, Culture, Sports, Science and Technology and inspection reports collected by the School Lunch Sanitation Promoting Committee were analyzed.

Results: Seventy-four outbreaks related to school lunches and 23,640 cases were reported in the 10 years. However, the number of outbreaks decreased from 18 in 1996 to less than 5 in 2004, and the cases also decreased, from 11,651 in 1996 to 401 in 2005. Deaths were reported only in 1996. The causative agents were mostly bacteria and the reported species were diverse prior to 1998. After 1998, norovirus appeared most frequently; (in ca. 60% of outbreaks). The School Lunch Sanitation Promoting Committee has inspected every facility where outbreaks occurred. From their reports, sources of contamination, opportunities for bacterial proliferation, cross-contamination processes, improper equipment, problems in crisis management and insufficient hygiene control of food suppliers were indicated.

Significance: Activities that have taken place since 1996 to improve school lunch sanitation were effective in reducing foodborne outbreaks due to school lunches in Japan. However, continuous inspections, analyses of causes of individual outbreaks, and critical review of the current systems are emphasized.

P3-16 Pulsed Field Gel Electrophoresis (PFGE) of Human and Meat Isolates of *Escherichia coli* O157:H7 for Source Attribution in International Trade

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Introduction: A potential common source outbreak of *Escherichia coli* O157:H7 with the PFGE *Xba*I:*Bln*I pattern of EXHX01.0074:EXHA26.0569 was reported by PulseNet USA in mid-2006. Contemporaneously, isolates with similar PFGE patterns (EXHX01.0074: EXHA26.0569 and EXHX01.1401: EXHA26.0569) were isolated under the USDA PR-HACCP monitoring program from two manufacturers of ground beef containing a mix of US and New Zealand (NZ) beef, the latter from the same slaughterhouse. Even though these PFGE profiles are common in the US, the US-CDC and USDA-FSIS independently requested from NZ authorities information on the occurrence of these patterns in NZ human and beef isolates.

Purpose: To describe the occurrence of *E. coli* O157:H7 isolates with *Xba*I:*Bln*I patterns of EXHX01.0074:EXHA26.0569 and EXHX01.1401:EXHA26.0569 in NZ human cases and from NZ beef.

Method: PFGE *Xba*I profiles were developed for 203 human isolates from notified confirmed cases of *E. coli* O157:H7 gastroenteritis, and 229 beef (adult and bobby calf) isolates collected from regulatory and commercial monitoring programs, and research surveys for the period 2003–2006.

Results: Although 12 human and 3 beef isolates had *Xba*I patterns indistinguishable from EXHX01.0074, none had EXHA26.0569 *Bln*I patterns. Similarly, none of the isolates had EXHX01.1401 *Xba*I patterns. Two beef isolates had EXHA26.0569 *Bln*I patterns but their *Xba*I patterns were dissimilar to those of the US isolates.

Significance: All NZ human and beef *E. coli* O157:H7 2003–2006 were genotypically dissimilar to those isolated from US ground beef and a suspected point source outbreak. It is therefore unlikely that NZ beef is the original source of the US ground beef or human case isolates.

P3-17 DSC Validation of Models for Proteolytic *Clostridium botulinum* Growth during Cooling of Cooked Ground Beef

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Clostridium botulinum is an anaerobic, spore-forming bacterium that causes botulism. USDA Food Safety and Inspection Service guidelines for cooling heat-treated meat and poultry products dictate certain cooling rates, but also suggest that computer modeling can be a useful tool in assessing the severity of cooling deviations. The purpose of this study was to validate the USDA Pathogen Modeling Program (PMP) models in cooked ground beef cooled at different rates. Validation was accomplished using dynamic (changing temperature) experiments as well as static (constant temperature) experiments. Ground beef inoculation was performed inside a Coy anaerobic chamber. Experiments were performed inside the chamber or in vacuum sealed bags. A spore cocktail with approximately equal amounts of six different proteolytic strains of *C. botulinum* was used to inoculate 75% lean ground beef for a final concentration of ~100 spores/g. All experiments were performed in duplicate. Samples were cooled from 54.4 to 4.4°C over 24 or 48 h during cooling experiments. Experiments to determine *C. botulinum* growth rates at eleven (11) different constant temperatures between 12 and 37°C were also performed. Differences between model predictions and actual growth of *C. botulinum* in ground beef were observed in every cooling experiment, with over-predictions ranging between 1.5 and 5.6 log CFU/g. In contrast, generally good correlations between *C. botulinum* growth rates and model predictions at constant temperatures were observed. These results show that the PMP is highly fail-safe for *C. botulinum* growth in cooked, cooled ground beef. These data also indicate that either unexpected physiological changes occur in *C. botulinum* during the cooling of ground beef, which cause errors in the model predictions, or else the dynamic model itself is incorrect and it erroneously translates constant temperature rates into growth rate predictions during changing temperatures.

P3-18 Yeast Growth-decay Modeling Using Logistic Function and Fermi Equation

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Most of the predictive microbial models focus on foodborne pathogenic bacteria, although spoilage fungi are also important, especially in foods with reduced a_w and pH, for which fewer models are available. Excel and Visual Basic were used to develop software that predicts yeast (*Saccharomyces cerevisiae, Debaryomyces hansenii, Candida krusei, Zygosaccharomyces bailii*) growth or decay within the range of 0.95–0.90 a_w, 4.5–3.5 pH, and taking into account the solute type utilized to adjust a_w (sodium chloride, glucose or sucrose) and the acid employed to adjust pH (acetic, phosphoric, citric or tartaric). Growth or decay curves were obtained from factorial design experiments and yeast behavior was modeled using non-linear regression with the logistic function (growth) or the Fermi equation (decay). Growth and decay regression parameters were utilized to adjust polynomial equations. Logistic function and Fermi equation were appropriate to describe yeast growth or decay, obtaining correlation coefficients greater than 0.95 and prediction errors lower than 10%. Polynomial equations include growth or decay parameters as a function of a_w, pH, solute and acid type as well as their interactions and were utilized

to predict yeast behavior in non-evaluated conditions. The developed software allows one to choose each of the studied variables (yeast, solute, acid, a_w, pH, and time) to predict microbial behavior in a user-friendly interface. Besides, the growth or decay is displayed showing the predicted parameters and an exportable time and yeast-count list. The user-friendly software is an excellent tool to predict and describe yeast behavior incorporating several important product formulation variables. The software program could also be used for educational and research purposes.

P3-19 Dose Response Survival Modeling of *Escherichia coli* Exposed to Simultaneous Application of High Frequency Ultrasound and Laurel and Coriander Essential Oils

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Selected models can describe the inactivation pattern of microorganisms exposed to lethal agents. Microbial population declines which occur as a result of exposure to lethal agents, applied individually or in combination, can be described by the Fermi function or the Weibull distribution. The main objective of our research was to evaluate and model the survival of *Escherichia coli* in a model system (tripticase soy broth, pH 5.5) exposed to the simultaneous application of high intensity ultrasound (US) and selected essential oil concentrations (laurel 0.500 or 0.250%; coriander 0.250 or 0.125%). US treatments (20 kHz, 95 μm-wave amplitude) were performed by introducing a 25 mm probe in a jacketed inoculated model system. The microbial populations of inoculated samples were examined by plate counting and spiral plate techniques during the treatments. In order to quantify the microbial response for the studied treatments, the Weibull type distribution for modeling resistance and the Fermi equation were applied. Model parameters were obtained using nonlinear regression. US treatment alone reduced *E. coli* 2-log in 9 min and was less effective than the simultaneous treatment with essential oils, which reduced *E. coli* more than 5-log cycles when using the highest evaluated concentrations. Experimental curves correlated well to predicted data (employing both equations), obtaining significant determination coefficients. US and US-essential oils survival curves exhibited a strong right skewed distribution with downward concavity. Fermi function predicted critical treatment times (50% reduction of initial population) that decreased with higher concentrations of the essential oils, demonstrating the effectiveness of combined treatments. The Weibull type distribution model and Fermi function were useful in explaining observed differences among treatments. The use of US combined with essential oils was effective in enhancing inactivation of *E. coli*.

P3-20 Protocol for Pasteurization Inactivation Kinetics of Milk Borne Pathogens

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Introduction: Raw milk in the farm vat is inevitably contaminated with a range of bacteria that include human and animal pathogens. Pasteurization has successfully addressed these hazards and its public health significance is unquestioned. In order to derive risk-based dairy foods standards, however, data on the extent of the pasteurization inactivation of various pathogens, as well as their incidence in raw milk, are required.

Purpose: The purpose is to develop a protocol for laboratory screening and pilot scale trials to derive kinetic data for predicting the heat inactivation of dairy pathogens by High Temperature Short Time pasteurization at 72°C/15 s.

Methods: A broad-based expert experimental design group evaluated key variables including species/strain selection, heating medium, screening method/temperature, pilot equipment scale and requirements, number of strains, and recovery media. The agreed protocol is being used for these experiments.

Results: Thirty representative strains of each species are being screened in a submerged coil apparatus and thermal inactivation kinetics calculated in an effort to identify the strains that were most heat resistant at 60°C. The most resistant strain will be used for pilot scale heat inactivation studies in UHT whole milk (4% fat) at 120 liter/h under turbulent flow over a range of temperatures with a 15 s holding-tube. Optimal non-selective media for maximum recovery of heat damaged cells will be used. Triplicate runs for each species using the same batch of UHT milk will assure consistency in experimental design and derivation of kinetic parameters.

Significance: The generation of kinetic, as opposed to "end point," data on the effect of pasteurization is essential for determining the level of protection offered by the process. A comprehensive and robust protocol for determining the extent of the pasteurization kill of key raw milk pathogens is critical for risk assessments and evaluation of alternative processing methods.

P3-21 Predictive Models for the Growth and Survival of Total *Vibrio parahaemolyticus* in Gulf Coast Shellstock Oysters

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Vibrio parahaemolyticus is a halophilic bacterium that occurs naturally in estuarine environments and can cause gastroenteritis in seafood consumers.

Gastroenteritis is usually associated with the ingestion of raw, undercooked, cross contaminated, and/or improperly stored seafood. Although there are models for the growth of *V. parahaemolyticus* in broth and post-harvest shellstock oysters, acceptable growth and survival models do not exist for the potential storage temperatures relevant to industry and consumer handing practices. The objective of this study was to develop predictive models for the growth and survival of total *V. parahaemolyticus* in shellstock oysters under commercially relevant storage temperatures. For this study, Gulf Coast oysters were collected from August though September 2006 and then stored at 5, 10, 20, 25, and 30°C for selected time intervals. At each time interval, two composite samples of six oysters each were analyzed separately by a direct plating method, using an alkaline phosphatase-labeled DNA probe targeting the species-specific thermolabile hemolysin (tlh) gene. The Baranyi D-model was fitted to the *V. parahaemolyticus* growth and survival data to define the parameters of lag phase duration (LPD), growth/inactivation rate (GR) and maximum population density (MPD). *V. parahaemolyticus* was slowly inactivated at 5°C at a GR of -0.003 log CFU/h. Maximum GR (1.5 log CFU/h) was observed at 25 and 30°C. At 10, 15 and 20°C, the GR were 0.014, 0.051 and 0.071, log CFU/h, respectively. The bias and accuracy factors for a square root secondary growth model were 1.00 and 1.02, respectively. MPD displayed a peak form, with a maximum of 7.9 log CFU/g at 20°C. No significant LPD was observed. The results of this study will assist risk managers and the seafood industry in designing more effective food safety systems.

P3-22 DSC Development of Predictive Mathematical Model for the Growth Kinetics of *Staphylococcus aureus* by Response Surface Model Based on Absorbance Data

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A response surface model was developed for predicting the growth rates of *Staphylococcus aureus* in tryptic soy broth (TSB) medium as a function of the combined effects of temperature, pH, and NaCl. TSB containing six different concentrations of NaCl (0, 2, 4, 6, 8, and 10%) was adjusted to six different initial pH levels (pH 4, 5, 6, 7, 8, 9, and 10) and incubated at 10, 20, 30 and 40°C. The primary growth curves were fit (R^2 = 0.9000 to 0.9975) to a Gompertz equation to obtain growth rates. The secondary response surface model for natural logarithm transformations of growth rates as a function of combined effects of temperature, pH, and NaCl was obtained using SAS general linear analysis. The predicted growth rates of *S. aureus* were generally decreased at basic (9, 10) or acidic (5, 6) pH levels or increased (0–8%) in NaCl concentrations. A response surface model was identified as an appropriate secondary model for growth rates on the basis of correlation coefficient (r = 0.9703), determination coefficient (R^2 = 0.9415), mean square error (MSE=0.0185), bias (Bf = 1.0216) and accuracy factors (Af = 1.2583). Therefore, our secondary model supported predictions of the combined effect of temperature, NaCl, and pH on growth rates for *S. aureus* in TSB medium.

P3-23 Probabilistic Modeling of *Enterobacter sakazakii* Survival to Dessication after Grown at Selected Water Activities

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Enterobacter sakazakii appears to have an unusual ability to survive when exposed to dry conditions. However, several strain differences are reported when studying the tolerance of the pathogen to osmotic and dessication stresses. The combined effects of a_w (0.99, 0.98, 0.97 or 0.96), solute utilized to adjust a_w (sodium chloride or glycerol), cell growth phase (samples taken after 2, 5, 9, 17 and 24 h of incubation), and dry incubation time (1, 2, 3 up to 7 days) were evaluated using two *E. sakazakii* strains (ATCC 51329 and one isolated from a soy bean milk product) for growth/no growth response. Also, threalose production was evaluated by HPLC analysis. Fifty mL of cell suspensions of each strain taken from the evaluated systems and selected incubation periods were deposited in microtiter plate wells. The inoculated wells were dried in a laminar flow hood (3 h), and incubated at 37°C. After every studied incubation period, 100 mL of tripticase soy broth was added and incubated at 37°C for 24–48 h. If growth was observed (turbidity change), the response was registered as 1; if no growth (no change in turbidity) was observed, the response registered as 0. Backward stepwise logistic regression was employed to develop a simplified model able to predict the probability of *E. sakazakii* growth. The model showed that cells in the stationary phase had higher probabilities of growth after drying and showed longer incubation times when grown in media with higher a_w adjusted with glycerol. The evaluated strains presented important resistance differences; this could be attributed to undetectable thealose production. The model obtained can be used to compare strain stress tolerance differences. Results provide insights to predicting survival characteristics of *E. sakazakii*.

P3-24 Modeling the Bacterial Survival/Death Interface Induced by High Pressure Processing

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Introduction: Many predictive models of microbial inactivation in high pressure processing (HPP) have been developed in recent years. However, most of these are kinetic models that do not take into account factors other than pressure and time. Furthermore, the predictive models do not take into account the recovery of injured bacteria induced by HPP. Therefore, novel predictive models of HPP-induced microbial inactivation should be developed, taking into account the effect of plural factors and the recovery of HPP-injured cells.

Purpose: The purpose of this study was to develop a survival/death interface model that takes into account the effect of multiple factors and recovery for prediction of inactivation of *Listeria monocytogenes* by HPP.

Methods: The model was derived from data sets comprising 360 combinations of environmental factors such as pressure (200, 300, 400, 500 MPa), pressure-holding time (1, 3, 5, 10, 20, 30 min), pH (3, 4, 5, 6, 7), and inoculum level (3, 5, 7 log CFU/ml). The survival and death of *L. monocytogenes* after HPP was determined by the presence and absence of colony forming ability on non-selective agar plates after 30 days incubation at 20°C in broth, taking into account the recovery of HPP-induced injured cells.

Results: The linear logistic model with time logarithmically transformed gave a degree of agreement between probabilities predicted by the fitted model and all observations, as 99.3% concordant. The model provided a good fit to the data as shown by performance statistics. The developed interface model provided requisite process conditions for the target effect of HPP on *L. monocytogenes*.

Significance: Optimization of HPP could be accomplished using the model developed in this study. Furthermore, choice of processing factors allows for processing flexibility in HPP and specifies the process criteria that are incorporated into the HACCP plan.

P3-25 Kinetic Model of Tropomyosin Denaturation for Predicting Inactivation of *Salmonella* in Cooked Beef

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Introduction: To ensure the destruction of *Salmonella* and other foodborne pathogens in cooked meat and poultry products, USDA-FSIS has established lethality performance standards for cooked, ready-to-Eat meat and poultry products. For regulatory and quality control purposes, analytical tools for assessing whether the lethality performance standard is met would provide further assurance of the safety of finished products.

Purpose: The purpose of this study was to evaluate a developed kinetic model describing the impact of heat processing on tropomyosin (TM) denaturation for the prediction of the effectiveness of the *Salmonella* inactivation in cooked beef.

Methods: Ground beef samples with 15% fat were inoculated with a cocktail of *Salmonella* strains at 10^8 CFU/g, and processed to endpoint temperatures of 50, 55, 60, 65 and 70°C with varied heating rates. Surviving *Salmonella* in the cooked samples was enumerated by surface plating on tryptic soy agar with 0.6% yeast extract and 1% sodium pyruvate. TM concentrations were measured by ELISA employing a monoclonal antibody against bovine TM, and the predicted values of TM in the cooked samples were derived from the kinetic model.

Results: Residual TM in beef samples after heating closely followed first-order kinetics. Integration of the equation with time-temperature profile enabled the prediction of TM in cooked samples. Percent errors between the values of residual TM measured by the ELISA and the values predicted by the kinetic model were less than ± 4.5%. The high limits of 99% confidence intervals were determined as 2430 and 2980 μg/ml of TM when 6.5-log reductions of *Salmonella* were achieved in the cooked samples at heating rates of 0.4 and 1.6°C/min, respectively.

Significance: The kinetic model provides an accurate and reproducible estimation of the effectiveness of *Salmonella* inactivation in cooked beef products under defined processing conditions.

P3-26 Uncertainty Assessment of Broth-based Microbial Growth Models

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Users of predictive models should understand how various sources of uncertainty and variability contribute to overall error. Otherwise, underestimation and/or overestimation can lead to significant problems in commercial food processing applications, ranging from unacceptable consumer risk to over-processed product. The objective of this study was to identify and quantify sources of uncertainty and variability in broth-based growth models. The total uncertainty of a model was assumed to be the aggregate contribution of errors due to organism, substrate, laboratory methodologies, replications, and primary and secondary regressions. The approach was tested using the Gompertz/response-surface growth models for *Listeria monocytogenes* from the USDA Pathogen Modeling Program. The total uncertainty was quantified as the root mean squared error between observed and predicted values. The experimental variability due to organism, substrate, and laboratory methodologies was neglected, because they were constants in the study. To compute the error due to replication, the data were classified into treatments, depending on experimental conditions (pH, nitrite, salt, and temperature), and a 2-way ANOVA was applied (factors: treatment and time). To compute the uncertainty from the secondary regression, the upper and lower confidence limits of the log predictions were calculated by applying the upper and lower limits of the Gompertz parameters. The total uncertainty was 1.35 and 1.62 log CFU/ml for aerobic and anaerobic conditions, respectively. For the same two conditions, the errors from the primary regression were 1.02 and 1.22 log CFU/ml. The errors from the secondary regression were 1.48 and 1.42 log CFU/ml, and the variability due to replications was 0.26 log and 0.21 log CFU/ml. Knowledge of the relative contribution of errors to the total uncertainty of a specific model can help prioritize efforts to minimize them and can enable the industry to more reliably apply predictive microbiology for risk assessment, process design, analysis, and validation.

P3-27 DSC Modeling the Risk of *Salmonella* in Raw Poultry as Influenced by Different Further Processing and Packaging Practices

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The regulations which govern the temperatures and procedures for chilling and freezing of ready-to-cook poultry (9 CFR, 381.66) state that eviscerated poultry to be shipped from the establishment in packaged form shall be maintained at 40°F. These same regulations also allow the temperature to rise to a maximum internal temperature of 55°F during further processing and packaging operations provided that immediately after packaging, the internal temperature of the poultry is promptly lowered to 40°F or less, or the product is frozen. The purpose of this study was to evaluate the public health risk posed by various further processing and packaging operations, including those that meet the regulations, those that exceed the regulations, and those that match actual industry practice. Computer models for *Salmonella* growth in raw poultry were combined with literature based assumptions about the prevalence and concentration of *Salmonella* in raw poultry. Various further processing and packaging operation time and temperature profiles, based on actual industry practice, were used to simulate pathogen growth on poultry. The risk posed by the simulated real-world further processing and packaging conditions (where processed product rises to 65°F and then is cooled from to 40°F in 6 h) was only 77% of the risk posed by holding the prduct at 54°F for an entire 8-h shift. The risk posed by product held at 54°F for 8 h was 67% of the risk posed by holding the product at 56°F for an entire 8-h shift. This risk posed by any of these regimes was less than the risk posed by *Salmonella* population increase as slaughtered chickens were cooled from poultry body temperature to 40°F.

These findings show that a risk-based approach to food safety can provide information useful in determining the risk posed by different poultry processing and packaging conditions.

P3-28 Development of a Kinetic Model for Mold/Yeast Growth on Seasoned Lotus Root Cuts for Online Shelf-life Control

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Online quality monitoring and management in the food supply chain are sought to minimize potential microbiological risk and ensure desired quality. Recently, time temperature indicator and radio frequency identification (RFID) tag systems with appropriate sensors have emerged as a tool to track the food distribution channel and demonstrate quality. However, incorporating this technology for microbial quality control on a real-time basis requires a kinetic model of microbial spoilage. The purpose of this study was to develop a simple microbial quality prediction model for predicting shelf life of Korean seasoned lotus root cuts, whose spoilage is due to growth of mold and yeast. Mold/yeast counts of Korean seasoned lotus root cuts were measured during storage under the constant temperature conditions of 0, 5, 10 and 15°C. The parameters of microbial growth of Baranyi & Roberts were determined using Box's complex method based on the mean microbial plate counts which were resampled from the experimental data by the bootstrapping method. The distribution of the model parameters was examined, and their temperature dependence was provided as mathematical functions for application to dynamic storage conditions whose temperature was monitored by an RFID system. While parameters of initial and maximum cell densities were independent of temperature, initial physiological state and maximum specific growth rate were a function of temperature. The temperature functions of the parameters could be successfully incorporated into the differential equations of microbial growth to predict the mold/yeast count under fluctuating temperature conditions. The developed kinetic model provides a basis for predicting shelf life of Korean seasoned ready-to-eat foods whose spoilage is attributed to growth of molds and yeasts.

P3-29 The International Risk Governance Framework as Applied to *Listeria monocytogenes* in Raw Milk Soft Cheese in the United States

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The International Risk Governance Council (IRGC) has proposed an integrated risk governance framework that entails the pre-assessment, appraisal, judgment, management, and communication of risks. This framework is novel in that it gives equal importance to social and biological aspects of risks. We have applied the framework to *Listeria monocytogenes* (Lm) in raw milk soft cheese (RMSC). The pre-assessment phase frames the issues pertinent to stakeholders, where two opposing frames are presented: Illness Prevention and Consumer Sovereignty. The risk appraisal phase involves both risk and concern assessments. Using the assessment information, risks are characterized and evaluated to develop risk reduction options, which are then assessed and implemented into risk management strategies. Currently, the retail sale of RMSC in the US is illegal. However, four alternative risk management strategies were identified based on the pre-assessment frames: (1) allow the retail sale of commodity specific RMSC; (2) require mandatory warning labels; (3) change the tolerance

levels from zero tolerance to 100 CFU/g; and (4) implement HACCP or best management practices. Each strategy would involve a different approach to risk communication. The framework is useful in that it: (1) facilitates the conceptualization of a range of stakeholders throughout the commodity system; (2) draws attention to risk decision-making at the regulatory level; (3) and forces management to integrate the social sciences into each step of the risk assessment. This process includes the evaluation of public perceptions and the identification of contentious issues surrounding RMSC, the conceptualization of risk management options, the estimation of uncertainty in risk assessment, and the determination of acceptability.

P3-30 Monte-Carlo Simulation to Predict the Effect of Multiple-sequential Interventions on Bacterial Populations during Poultry Processing

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Introduction: Quantitative microbial risk assessment for food assists in determining levels of microorganisms that may pose a health risk to consumers as well as ways to improve practices in the food chain that reduce exposure to acceptable limits.

Purpose: The purpose of this study was to create a mathematical model to predict the effect of multiple-sequential interventions on bacterial populations on poultry carcasses in a commercial processing facility.

Methods: Multiple-sequential interventions evaluated at the processing facility included: New York wash, post-evisceration wash, inside/outside bird wash 1 (IOBW1), IOBW2, chlorine dioxide wash, chlorine dioxide/chlorine chiller, chiller-exit spray, and post-chiller wash. Carcass samples were collected before (n = 75) and after (n = 75) each intervention and analyzed for aerobic plate counts (APC), total coliform counts (TCC), and *Escherichia coli* counts (ECC). The data for each individual intervention was fitted to a triangular distribution with parameters minimum, maximum, and mode. The adequacy of the fitted distribution was verified by performing a chi-square goodness-of-fit test on the observed and expected quantiles generated from the data for each intervention. Simulations for each intervention were run with the underlying distribution parameters using Monte-Carlo sampling for 50,000 iterations. Probability plots were generated for log survivors following each intervention.

Results: With the intervention system evaluated, the generated model indicated that birds entering the facility with mean TPC of 4.5 log CFU/ml have a 57% likelihood of exiting the post-chiller wash with counts of 2.0–3.0 log CFU/ml. Birds entering the facility with mean TCC and ECC of 3.0–3.5 log CFU/ml are 96% likely to meet the microbiological criteria (≤ 2 log CFU/ml) for broilers (PR/HACCP Final Rule, 1996).

Significance: The model developed for this intervention system may be used by the processor to evaluate the food safety system and predict the effect of adding or removing interventions from the system.

P3-31 Quantitative Risk Assessment in HACCP for Safe Menu in Korean Restaurants: Focus on Vegetable Dishes (Sangchae)

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The Korean vegetable dish Sangchae is common in Korean cuisine. It has the potential to cause food poisoning in Korean restaurants due to its nature. This study was carried out to develop a Quantitative Risk Assessment (QRA) model for *Staphylococcus aureus* in Sangchae and establish the specifications for its risk management. The processes for preparing Sangchae consist of four steps from pre-treatment to final consumption. The uncertainty and variability in the data were treated with Monte-Carlo simulation. Sensitivity analysis showed that the storage time and temperature in restaurants were the critical control points. It was suggested that temperature control (under 10°C) was the most critical condition to prevent growth of *S. aureus* in Sangchae. Based on this study, QRA was a beneficial tool for evaluating factors influencing potential risk and could be applied directly to risk management.

P3-32 Fuzzy Math Calculation for Quantitative Risk Assessment on Korean Vegetable Dishes (Sangchae)

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Fuzzy math was applied to quantitative risk assessment on the Korean vegetable dish Sangchae, compared with Monte Carlo simulation. Microbiological risk factors associated with *Staphylococcus aureus* for Sangchae were identified, and exposure assessment was performed for four steps from pre-treatment to final consumption. Microbial assays were made of raw materials and kitchenware. Fuzzy values of the microbial data first were established and the fuzzy ¥á-cut method was applied in operations. In Monte Carlo simulation, the microbial data first were fitted to various probability distribution functions, then optimum functions were used in iterative calculations. Consequently, the estimates from fuzzy math calculations were similar to those from Monte Carlo simulation in terms of accuracy, whereas variability of fuzzy estimates was larger, while calculation time was shorter. Based on this study, fuzzy math calculation was useful to estimate data in a quantitative risk assessment of Sangchae.

P3-33 DSC Quantification of *Salmonella* from Pig Feces by Real-time PCR

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We have developed a protocol based on real-time PCR to quantify *Salmonella* DNA in pig feces. Microbial DNA is directly purified from fecal material taken rectally based on an optimized protocol of the QIAamp Stool Kit (Qiagen). PCR inhibitor-free DNA is quantified in a *Salmonella* specific real-time PCR targeting the *ttr*-locus. Artificially inoculated *Salmonella* cells in pig feces could be recovered at a rate between 20% and 40% depending on initial number of cells. DNA purified from feces artificially inoculated with *Salmonella* cells (serially 10-fold diluted cells) was used for the establishment of the standard curve. A correlation coefficient of > 0.994 and an efficiency between 0.9 and 1.0 indicates an accurate quantification over a five log range down to 6.5 genomic copies per PCR (650 genomic copies per gram feces). An "in-house" validation study, using 186 feces samples of 13 pigs infected with *Salmonella* Typhimurium DT104 (nalidixic acid resistant) demonstrated a good agreement between real-time PCR and direct plating on XLD agar containing 50 μg/mL nalidixic acid. This real-time PCR-based *Salmonella* quantification method is a new alternative to estimate the levels of *Salmonella* contamination in a swine herd as well as to provide easier quantitative data for risk assessment models. It has also the potential to quantify *Salmonella* in food matrices.

P3-34 DSC Comparison of Quantitative, Real-time PCR to Most Probable Number Method for *Salmonella* Typhimurium DT104 from Swine Feces

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Introduction: *Salmonella* Typhimurium is a ubiquitous foodborne pathogen that is commonly isolated in swine production and processing, with phage type DT104, and is particularly important because of its high spectrum of antibiotic resistance. Development and validation of rapid methods for identification and quantitative analysis of this organism would aid in surveillance and prevention of transmission of antimicrobial resistant strains of *Salmonella* associated with contaminated pork.

Purpose: The purpose of this study was to compare a quantitative real-time PCR (qPCR) and a most probable number (MPN) method for detection and enumeration of *S.* Typhimurium DT104.

Methods: A standard, produced by amplifying *S.* Typhimurium DT104 DNA with the blaPSE1 primers, was used to construct a qPCR standard curve. DNA was extracted from feces obtained from previously challenged pigs and followed by qPCR amplification. Simultaneously, feces were serially diluted from 1 to 0.01 g, inoculated into tetrathionate broth for enrichment, followed by culture on XLT4 agar. Presumptively positive colonies were confirmed by standard biochemical tests, and the number of colony forming units (CFU) determined by a MPN calculator.

Results: The detection limit of the qPCR assay was 1.0×10^3 genome copies/g feces. qPCR and MPN methods were correlated based on graphical trends. Fecal shedding of *S.* Typhimurium ranged from >110 to < 0.3 CFU/g as calculated by MPN and 2.72×10^7 to $< 1.0 \times 10^3$ genome copies/g as determined by qPCR. The qPCR method provided a greater range of quantitative detection than did the parallel MPN assay.

Significance: These data suggest that qPCR can accurately quantify fecal shedding of *Salmonella*, particularly when the organism is present in high copy number. Efforts continue to refine the qPCR method to improve the lower limit of detection and to apply the assay to *Salmonella* detection and enumeration in other complex sample matrices such as meats and eggs.

P3-35 Reverse-Transcriptase-PCR for the Rapid Detection of *Salmonella* Using *invA* Primers

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Introduction: Recent outbreaks of *Salmonella* linked to fresh produce emphasize the need for rapid detection methods to help control outbreaks. Application of real-time PCR to foods remains challenging, especially for the detection of viable pathogens. Reverse-transcriptase-PCR (RT-PCR) detects the presence of mRNA (shorter half-life than DNA), with greater potential of detecting viable pathogens. The chromosomally located *invA* gene of *Salmonella* is required for host invasion and is widely used for the detection of this pathogen by PCR.

Purpose: Detection of *Salmonella* was undertaken using newly designed primers based on the *invA* gene by RT-PCR and compared to real-time RT-PCR (rt-RT-PCR).

Methods: *Salmonella* serovars Typhimurium and Enteritidis were grown (7.68 log CFU/ml) in Luria-Bertani broth (LB) at 37°C for 24 h, and RNA was extracted. Newly designed primers based on the *invA* gene (347 bp) were used in RT-PCR and rt-RT-PCR reactions, without and with SYBR green I, respectively (BioRad iCycler), on serially diluted RNA, followed by gel electrophoresis. All experiments were replicated at least twice.

Results: Detection for both serovars using RT-PCR was lower (-2-log diluted RNA, ~5 log CFU/ml) than rt-RT-PCR (-4-log diluted RNA, ~3 log CFU/ml) by gel electrophoresis. Melt curve analysis showed Tm at 87.5°C with Ct values from 16–29 for up to 3 log CFU/ml.

Significance: Rapid detection of *Salmonella* by rt-RT-PCR eliminates the need for gel electrophoresis with improved detection as compared to RT-PCR. Experiments with heat-inactivated overnight cultures of *Salmonella* are being carried out to compare detection to viable *Salmonella*. Assay validation is ongoing to determine absence of cross reactivity against a panel of foodborne bacterial pathogens. Furthermore, optimization of the rt-RT-PCR and improved RNA extraction strategies should allow detection sensitivity to increase. This method has tremendous potential to be applied to foods.

P3-36 Immunomagnetic Chemiluminescence (IMC) Rapid Detection of *Listeria* spp.

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Listeria is one of the emerging food pathogens of which public awareness has increased as food safety concerns broaden. The current detection methods for *Listeria* require timely procedures (i.e., pre-enrichment, selective enrichment, selective plating and genetic confirmation), which may take 2–3 days to have the results confirmed. Considering the fast pace of the food industry, there is a strong need for a near-real-time detection method for the presence of *Listeria* in the food processing environment. PDX-Immuno-Magnetic Chemiluminescence *Listeria* (PDX-IMC-List) is a unique, no enrichment, rapid screening assay for the detection of *Listeria* spp. in environmental samples. PDX-IMC-List is based on immunoselective capture of *Listeria* cells from an environmental sample by the help of magnetic beads coated with *Listeria*-specific antibodies and allowing immunocaptured cells to react with a *Listeria*-specific chemiluminescent enzyme substrate. Performance of PDX-IMC-List has been tested in five different categories: inclusivity-exclusivity, method comparison, ruggedness, combined lot-to-lot variability and shelf life, and real environmental samples from food processing facilities in the US. Inclusivity-exclusivity data suggested that PDX-IMC-List had 100% sensitivity and 96.7% specificity. Based on the chi square values, method comparison studies suggested that there were no significant differences between the reference USDA method and PDX-IMC-List assay. Results of ruggedness studies suggested that instructions for use parameters needed to be followed carefully with volumes of test reagents being the most articulate. No significant difference was seen among three production lots after up to 2 months of refrigerated storage. Studies conducted with naturally contaminated environmental samples (n = 327) showed that PDX-IMC-List had only 1% false negative and 8% false positive results. When PDX-IMC-List was used as a screening assay for environmental samples from a food processing facility, a 50% savings in further pathogen confirmation was achieved.

P3-37 Affinity Magnetic Separation, a Novel Sensitive and Specific Method Drastically Reducing the Time of Enrichment for *Listeria*

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One of the major foodborne pathogens, *Listeria monocytogenes*, is difficult to control due to its ubiquitous presence in food processing environments at low numbers and its ability to adapt to environmental stress. The natural properties of *Listeria monocytogenes* create a need for sensitive test methods in order to ensure food safety. In this study we evaluated the time-saving benefits and sensitivity of Affinity Magnetic Separation (AMS), a method based on bacteriophage derived *Listeria* Binding Protein. AMS enables the combination of specific cell capture with any subsequent detection procedure ranging from nucleic-acid based methods to viable cell count. The initial step is the attachment of biotinylated *Listeria* Binding Protein to *Listeria* present in the pre-enriched food sample. In a second step, streptavidin coated magnetic beads are added to the sample and, after a short incubation at room temperature, the captured *Listeria* cells are separated from the sample using a large-volume magnetic separator. In order to analyze the detection limits of the method described, seven different food types were naturally contaminated or inoculated with low levels (1.0–12.0 CFU/25 g) of *Listeria* spp. to (1) measure growth rates of *Listeria* in different food enrichments, 2) estimate the detection limit of AMS and 3) assess the minimum time needed for pre-enrichment using AMS. Theoretical and observed growth rates of *Listeria* were compared and variation depending on food type was confirmed. The detection of *Listeria* using AMS required the presence of 1.0–4.0 CFU/ml. At such low cell numbers, 60–90% of the *Listeria* were captured. Depending on food matrix, the time needed for pre-enrichment was estimated to 6–8 h. In summary, our data demonstrate that AMS improves the overall test sensitivity and the pre-enrichment time can be reduced down to 6–8 h to ensure the detection of one initial cell in 25 g food sample.

P3-38 Characterization of an Affinity-purified Antibody Specific for *Listeria* spp.

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Listeria bacterial species are ubiquitous in the environment, in the soil and on plants and animals. Among them, *Listeria monocytogenes* is the major foodborne pathogen that causes human listeriosis. Listeriosis is associated with consumption of contaminated food products. A high mortality rate of infection prompted the government to establish standards for detection of *Listeria* spp. in Ready-to-Eat foods. Immunoassay systems are efficient methods for early detection of *Listeria* spp. in order to fulfill these regulatory needs. However, rapid detection of *Listeria* using immunoassays is highly dependent on antibodies with a high degree of sensitivity and specificity for the organism. We have developed a highly specific polyclonal antibody for *Listeria* spp. for this purpose. The *Listeria* spp. antibody was compared to other commercially available antibodies and demonstrated high reactivity to all six *Listeria* species, as well as low cross-reactivity against closely related Gram positive organisms, including *Enterococcus* spp., *Streptococcus* spp., *Lactococcus* spp., and *Staphylococcus* spp. The antibody is capable of detection of greater than or equal to 10^4 CFU/ml of *Listeria* cells. The results indicate that this antibody has excellent sensitivity and specificity towards detection of *Listeria* spp. following primary enrichment of suspect samples.

P3-39 Evaluation of Cationic Magnetic Separation Beads for the Capture of *Escherichia coli* O157:H7

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Introduction: Verotoxigenic *Escherichia coli* (VTEC) are a major cause of foodborne illness. Immunomagnetic separation (IMS) beads targeting the O antigen are limited in their application to a small proportion of VTEC serotypes, such as *E. coli* O157. Magnetic separation beads that capture microorganisms on the basis of non-serological surface properties might be an alternative for simultaneously recovering a wider range of VTEC from foods.

Purpose: The purpose of this study was to compare the ability of cationic magnetic separation (CMS) and IMS beads to capture *E. coli* O157:H7 from liquid or ground beef samples.

Methods: The ability of CMS beads to capture VTEC from liquid or ground beef samples was assessed using the Pathatrix system (Matrix MicroScience, Golden, CO) in triplicate experiments. Samples of Buffered Peptone Water (BPW) (25 ml) or ground beef (25 g) were inoculated with *E. coli* O157:H7. Ground beef was sampled with CMS and IMS beads. Without pre-enrichment, samples were mixed with 225 ml of BPW and circulated though the Pathatrix system for 30 min at 25°C. After washing, recovered beads were spread plated onto sorbitol MacConkey agar. To determine if pH altered the affinity of the beads for cells, CMS beads were suspended in PBS at pH 7.2 and pH 4.0.

Results: The lowest concentration of cells in BPW that could be reliably captured (3/3 trials) by CMS was 5.03 log CFU/ml. In ground beef, 5.88 log CFU/g for CMS and 1.82 log CFU/g for IMS, in the presence of a total aerobic count of 4.75 log CFU/g. Cells were found to be released from CMS beads in PBS at both pH 7.2 and pH 4.0.

Significance: These experiments show that CMS beads have a low affinity for *E. coli* O157:H7, though background flora did not interfere with capture from ground beef.

P3-40 Rapid Detection of *Escherichia coli* O157:H7 in Minced Beef Meat Using Nucleic Acid Sequenced-based Amplication Technology

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Introduction: *Escherichia coli* O157:H7 is an important emerging foodborne pathogen. It has been associated with bloody and non-bloody diarrhea, but more serious complications such as hemorrhagic colitis and haemolytic-uremic syndrome (HUS) can occur. Traditional and standardized analysis of food for the presence of this pathogen relies on methods that are laborious and time consuming. The molecular Nucleic Acid Sequenced-based Amplication (NASBA) method provides a rapid alternative for detecting *E. coli* O157:H7 from food since only 1.5 h is required for amplification and detection.

Purpose: The aims of our study were to investigate the specificity and sensitivity of the NASBA protocol, and to evaluate its performance in detecting *E. coli* O157:H7 in experimentally inoculated minced beef.

Methods: Eighty-one isolates consisting of STEC, *E. coli* O157:H7, non-toxigenic *E. coli* and non *E. coli* were tested by NASBA to evaluate the specificity of the NASBA method. The NASBA's sensitivity was determined using 3 strains of *E. coli* O157:H7. Moreover, the performances of this new technology was checked for detecting *E. coli* O157:H7 from minced beef experimentally inoculated.

Results: Specificity reached 100% and the detection limit was between 10^3 and 10^4 CFU/ml in pure culture. Moreover, the NASBA technology was able to detect *E. coli* O157:H7 inoculated at a level of 1 to 4 CFU/25 g of minced beef in only 8 h of enrichment.

Significance: This study has emphasized that NASBA is a new, promising rapid method for detecting foodborne pathogens from food that gives results in less than one day.

P3-41 Detection of Enterohemorrhagic *Escherichia coli* O157 and O26 and Food by Plating Methods and LAMP Assay: A Collaborative Study

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Introduction: Numerous infections with the enterohemorrhagic *Escherichia coli* (EHEC) serotype O26 have occurred worldwide, although serotype O157 is the most major serotype of the pathogen. However, there are few effective detection methods to isolate serotype O26.

Purpose: The purpose of this study was to evaluate the utility of a molecular detection method using a LAMP assay and culture methods in combination with immunomagnetic separation (IMS) and plating onto agar media for the detection of *E. coli* O157 and O26 in food.

Methods: Different laboratories participated in the study by conducting the trials to detect *E. coli* O157 and O26 from artificially inoculated ground beef and alfalfa sprout samples, using the same detection procedure. Twenty-five g of each sample were added to 225 ml of modified EC broth with novobiocin and then incubated at 42 ± 1°C for 20 ± 1 h. The culture was streaked onto selective agar media directly and after IMS, and also tested by the LAMP assay for the Verocytotoxin (Shiga Toxin) gene.

Results: *E. coli* O157 was detected in most samples of ground beef and alfalfa sprouts by the LAMP assay, the direct plating and the IMS-plating methods. *E. coli* O26 was detected in approximately 100% of the food samples by the LAMP assay. However, the direct plating and IMS-plating methods recovered 50 and 80% in ground beef samples, respectively. The results suggest that the LAMP assay is superior to the IMS-plating method.

Significance: Based on these results, it appears the LAMP assay is effective as a screening assay to isolate *E. coli* O157 and O26 from food samples.

P3-42 A Novel Most-Probable-number Plate Developed for Use with the 5-tube MPN Table

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Introduction: The most-probable-number (MPN) method is useful for estimating the number of bacteria in a sample. We have developed a MPN plate for the enumeration of coliforms that can be used with a 5-tube MPN table.

Purpose: The purpose of this study was to evaluate a novel MPN plate developed for the enumeration of coliforms in a sample.

Methods: Sterilized water was inoculated with *Escherichia coli* ATCC 11775 to give between 2–1600 MPN/100 ml. One-hundred ml of this sample was added to EC Blue 100 (granulated Chromogenic powder medium for 100 ml in a special plastic bottle sterilized by electron beam). After the powder was completely dissolved, the mixture was inoculated onto the MPN plate. The original sample and two 10-fold dilutions were used for 5-tube-method with EC Blue 10 for 10 ml. Growth of *E. coli* was indicated by the development of blue color after incubation at 35°C for 24 h. The MPN was determined for both methods from the 5-tube MPN table. Results were calculated as log MPN/100 ml. The slope and intercept values were determined by linear regression analysis. The statistical analysis was performed using ANOVA.

Results: The mean log MPN with standard deviations in 64 samples was 1.87 ± 0.70 by the MPN plate and 1.89 ± 0.74 by the 5-tube-method. These data were not significantly different using ANOVA ($P > 0.05$). The correlation coefficient, slope, and intercept between the MPN plate and 5-tube-method was 0.97, 0.92, and 0.13, respectively.

Significance: These data suggest that the MPN plate may be useful for determining the MPN without the need for the traditionally labor intensive dilution procedures and multiple tubes.

P3-43 Detection of *Shigella* spp. Using a Selective and Differential Chromogenic Plating Medium

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A selective and differential plating medium (R & F *Shigella* spp. Chromogenic Plating Medium) (SSPM) has been developed that differentiates presumptive colonies of *Shigella* species (*S. sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*) from other related gram-negative enteric bacteria. The mechanism of differentiation used in SSPM involves a series of positive color reactions produced only by the non-*Shigella* spp., whereas *Shigella* spp. are defined by a series of negative reactions. This differentiation is accomplished by a combination of carbohydrate substrates in a growth medium containing phenol red and chromogenic and inducer substrates that are fermented and/or cleaved only by the non-*Shigella* spp. with color production by the *Shigella* spp. remaining inactive. On SSPM after 24–28 h at 35–37°C, the four *Shigella* spp. appear as clear to white colonies, whereas other gram-negative enterics appear on a plate as yellow, yellow-green, green, or blue colonies clearly differentiating them from the *Shigella* spp. On SSPM, 34 pure culture strains of *Shigella* spp. appear as clear to white colonies 1–3 mm in diameter without a surrounding precipitate in 24–28 h at 35°C. *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella enterica* serovars Derby and Dusseldorf, and *Klebsiella pneumoniae* appear, respectively, as yellow/yellow-green, green, yellow, and green colonies. Additional selectivity of SSPM is evidenced by the lack of growth of common species of five gram-positive genera (*Bacillus*, *Staphylococcus*, *Lactobacillus*, *Pediococcus* and *Enterococcus*).

P3-44 DSC Isolation and Infection of Potential Foodborne Viral Pathogens

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Introduction: Foodborne illness cases attributed to viruses are likely underreported due to difficulties of detection from foods. Pathatrix (Matrix Microscience) allows for rapid detection of viruses from large food samples by recirculation through immobilized cationic beads.

Purpose: This study assessed the use of cationic beads for the initial isolation followed by virus infectivity of bound and released virus. Food samples include Ready-to-Eat foods that have been involved in foodborne outbreaks.

Methods: Salsa or a representative food sample (25g + 225 mL water) was inoculated with virus from 3 representative families (*Caliciviridae*, *Poxviridae*, *Picornoviridae*) and analyzed with Pathatrix, using cationically-charged paramagnetic beads. After sampling, the beads were treated with a basic elution buffer (3% beef extract) or a 0.1N HCl solution to release the virus. Presence of the virus attached to and released from the beads was shown by PCR and virus cell culture infectivity by $TCID_{50}$.

Results: Both bead treatments (basic elution buffer and HCl solution) had a positive effect on the release of the virus, as shown by PCR for hepatitis A, Aichi virus and feline calicivirus. From salsa samples, 100% recovery (10^9 initial inoculum) of two picornoviruses (hepatitis A and Aichi) was observed from cell culture infectivity, and for both viruses ≥ 50% (4.6–5.6 log) virus bound was released after incubation in the basic elution buffer. In the HCl-solution more hepatitis A was released from the beads (6.1 log) compared to Aichi (5.3 log). The large pox virus did not bind as effectively to the cationic beads (5 log recovered from 10^7 initial inoculum).

Significance: This data suggests that foodborne viruses can be removed from the cationic beads for use in cell culture. Unlike traditional PCR methods,

cell culture analysis of the treated cationic beads allows for the determination of the active virus present in foods.

P3-45 Withdrawn

P3-46 Universal M13 Tailed Primers for Use in Sequencing PCR Products with Degenerative Primers or Short Amplicons

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Norovirus and Hepatitis A (HAV) are recognized as leading causes of foodborne illnesses in the United States. Advancements in research have lead to the development of rapid and sensitive molecular techniques for the detection of both enteric viral pathogens. DNA sequencing is generally considered the gold standard for accurate viral genotyping in support of epidemiological investigations. Unfortunately, due to the genetic diversity of noroviruses, degenerative primer sets are often used in RT-PCR for the detection of these viruses. As is the case of both HAV and norovirus, some cDNA amplicons produced by TaqMan style assays are generally small (90–125 bp) and are not suitable for direct sequencing. Therefore, cDNA fragments are generally cloned prior to sequencing, which is both time and labor intensive. In order to overcome these obstacles, norovirus and HAV primers were each tailed with the universal M13 forward and reverse primers. This modification adds to the overall sequenced product size and allows for direct sequencing of the amplicons utilizing complementary M13 primers. In this present study, 8 samples were analyzed using this method and multiple alignments of the sequenced samples revealed ≥ 85 nucleotide identities among the norovirus or HAV products tested. Tailing with M13 primers offers an alternative to cloning and allows for more accurate and direct sequencing of cDNA products produced by RT-PCR assays.

P3-47 *Enterobacter sakazakii* Can be Detected Simultaneously with *Salmonella* Using a Chromogenic Agar Plating Medium

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Introduction: *Enterobacter sakazakii* (Es) has been associated with morbidity and mortality in infants, transmitted though infant milk formula (IFM). It has been recommended that methods be developed for improved isolation of Es and also *Salmonella* from IFM and related matrices. During a more general study of foods, Es was found to grow well on CHROMagar *Salmonella* (CS).

Purpose: The aims of this study were evaluate isolation of Es, as well as the simultaneous detection of *Salmonella* and Es, on CS.

Method: The colony morphology on CS of a collection of clinical and food Es isolates as well as a range of *Enterobacteriaceae* was determined. Growth in various enteric enrichment broths and detection on CS were determined for Es and *Salmonella*, alone and in co-culture, with or without food matrix.

Results: All Es isolates produced 1–2 mm dark ('Prussian') blue colonies after 24 h at 37°C with development of a diffuse purple halo around the colony between 24 and 48 h. Some related enteric bacteria were similarly pigmented, but only after 48 h. Both Es and *Salmonella* grew well in BPW, EE, RV and TT broths, alone or in co-culture. Both organisms were recovered on CS after pre-enrichment in BPW and selective enrichment in RV and TT from a range of co-inoculated foods, including IFM. With a limited number of samples, this method was slightly better than that advocated by the FDA for detection of Es.

Significance: The results indicate that CS is suitable not only for the isolation of *Enterobacter sakazakii*, but also for the simultaneous detection of Es and *Salmonella* from selected foods using enrichment protocols designed for the latter bacterium. Use of the same enrichment and a single plating medium greatly facilitates the screening of high-risk food products such dairy powders and their derivatives for two significant pathogens.

P3-48 DSC A Comparison of RNA Versus DNA-based Amplification Methods for the Discrimination of Viable from Non-viable Bacterial Cells

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Introduction: Although nucleic acid-based foodborne pathogen detection strategies offer promise, a consistent concern has been their poor correlation to bacterial cell viability, largely due to long-term persistence of DNA even after cell death. Evidence has suggested that RNA may be a better target than DNA for detection of viable cells.

Purpose: The purpose of this study was to compare a real-time RNA amplification method (nucleic acid sequence-based amplification or NASBA) to a similar real-time DNA-based PCR method with respect to the ability to detect viable bacterial cells. *Salmonella enterica* serovar Typhimurium was chosen as the model, and detection limits of the assays were compared both before and after heat inactivation of the pathogen.

Methods: Ten-hour (late log phase) cultures of *S.* Typhimurium were subjected to different time and temperature conditions (no heat, 5 min at 60°C, 20 min at 60°C, and 15 min at 121°C) followed by immediate isolation and purification of both DNA and RNA. Real-time PCR and NASBA (using an mRNA target) assays, which were based on a fluorescent molecular beacon detection platform (bioMérieux, Lyon, France), were optimized to establish definitive detection limits and then applied to serially diluted nucleic acid extracts obtained from the untreated (control) and heat-treated *S.* Typhimurium cultures.

Results: For RNA extracted from live cells (10^8 CFU/ml) and those that had been heat treated for 5 min at 60°C (which resulted in a 4 log inactivation) and 20 min at 60°C (complete inactivation), NASBA

detection limits were similar. However, there was a 2 log drop in detection limits when NASBA was applied to the RNA obtained from autoclaved cells of S. Typhimurium. Real-time PCR detection limits were equivalent for DNA extracted from all samples, regardless of heat treatment.

Significance: The RNA-based amplification assay was more indicative of bacterial cell viability than was a parallel DNA-based amplification assay, suggesting that RNA may be a more reliable target in this regard. Further studies are underway to compare RNA versus DNA targets with respect to detection of viable and non-viable cells present in representative food matrices.

P3-49 Novel Method for Concentrating Bacteria from Biological Liquid Samples for Direct Real-time PCR Detection (No Pre-enrichment)

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Introduction: The presence of pathogenic organisms in food and the environment is a constant theat to public health. Foodborne pathogen testing is a major focus of food industries. In order to reach low level of detection, classical methods require up to one week that includes a significant amount of time spent on culturing bacteria. A major hindrance to direct (no pre-enrichment) detection are low pathogen counts, the presence of inhibitors and other extraneous materials.

Purpose: To increase sensitivity and reduce the time for pathogen detection, the study was designed to develop a direct (no pre-enrichment) method for efficient bacteria isolation from poultry rinses followed by rapid real-time PCR analysis.

Methods: We designed an integrated device that filters particulate materials and has membranes to capture and concentrate the pathogens from large volume of biological liquid samples such as chicken rinse (that is widely used in poultry testing). We recently developed a multiplex four-color real-time PCR assay for detection of *Campylobacter* pathogen strains. This assay was used for bacterial enumeration.

Results: *Campylobacter jejuni* was detected in real chicken rinse samples with high sensitivity. Significant Ct shift (~10 Ct) was observed when samples were tested before and after concentration using our newly developed filtration and capture procedure. This shift corresponds to about 1000 fold of enrichment. The protocol exhibited highly efficient removal of inhibitors as evidenced by improved Ct values for the internal positive control.

Significance: The method enables concentration of pathogens in chicken rinses with efficient removal of inhibitors that improves sensitivity. The direct (no pre-enrichment) sample preparation combined with real-time PCR analysis provided more precise quantitation results compared to enrichment protocols.

P3-50 Isolation of *Vibrio vulnificus* by Immunomagnetic Separation Using Anti-H Monoclonal Antibodies

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Introduction: *Vibrio vulnificus* is considered one of the most lethal of all human pathogenic vibrios, accounting for 95% of all seafood related deaths in the United States. One approach to improve the recovery and detection sensitivity of *V. vulnificus* without sacrificing assay time is though the use of immunomagnetic separation (IMS).

Purpose: To develop and optimize an IMS protocol using anti-H antibody for the concentration of *V. vulnificus* from phosphate buffered saline (PBS) suspensions.

Methods: Six monoclonal antibodies were produced by immunizing mice at 2 week intervals by injection of 50 μg of purified *V. vulnificus* ATCC 27562 flagellin. Murine spleen cells were collected and fused with myeloma cells for hybridoma production. Antibodies were purified from cloned hybridomas secreting anti-H IgG and analyzed by ELISA, using *V. vulnificus* polar flagellar cores bound to microtiter plates. Antibody exhibiting a high anti-H titer was then coated onto sheep anti-mouse IgG immunomagnetic beads (IMB) at a concentration of 4 μg/10^7 beads. The antibody-coated IMB were mixed with *Vibrio* cell suspensions on slides and coagglutination reactions observed. To determine binding capacity, 10^7 IMB were coated with 1–10 μg monoclonal IgG and incubated with 10^2–10^3 *V. vulnificus* cells/ml PBS on a shaker at 25°C for 30 min. After magnetic separation, the aspirated supernatant fluid was spread onto agar plates and incubated overnight, and after which the number of unbound *V. vulnificus* was determined.

Results: Starting with 25 g IgG, ELISA anti-H titers ranged from 128 to >2048. The anti-body-coated IMB coagglutinated 70 (100%) of *V. vulnificus* clinical and environmental strains within 30 s and did not react with 9 different *Vibrio* spp., including 42 *V. parahaemolyticus* strains. IMB coated with antibody bound between 20-40% of the bacteria at a concentration of 10^2–10^3 *V. vulnificus*/ml.

Significance: An IMS method utilizing anti-*V. vulnificus* H antibody would enhance the recovery and detection of this pathogen from environmental sources.

P3-51 Comparison of Real-time PCR with Conventional Culture for Detection of *Yersinia enterocolitica* in Environmental Swabs

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Introduction: Environmental monitoring can be useful for identifying sources of contamination and for evaluating efficiency of cleaning procedures. However, more work needs to be done on comparison of various environmental monitoring methods for optimum recovery of contaminants.

Purpose: The objective of the study was to compare the efficiency of quantitative real-time PCR and conventional culture for recovery of the foodborne pathogen *Yersinia enterocolitica* (YE) from environmental swabs of stainless steel surfaces.

Methods: Approximately 6 log CFU of stationary phase YE cells were spotted onto stainless steel plates, dried, and stored at 25°C for 0, 5, 24 and 48 h. Triplicate spots were sampled at each time point. Cells were recovered by swabbing each spot with a hydrated Speci-Sponge® 5X bi-directionally. After stomaching the swabs, viable cell numbers were determined from eluates by direct plating on tryptic soy yeast extract agar. DNA was extracted from eluates and tested for the presence of a YE-specific 16S rRNA gene fragment by quantitative real-time PCR, using a standard curve of genomic DNA for reference.

Results: As determined by direct plating, numbers of viable YE cells in swab eluates dropped from 6 log CFU/ml to 3 log CFU/ml within 5 h and were below the detection limit by 24 h. The real-time PCR assay quantified YE 16S rRNA genome equivalents within 1 log of the initial level for all replicates at all time points though 48 h.

Significance: Detection and quantification of YE DNA from environmental swabs by real-time PCR was demonstrated. Compared to conventional culture, real-time PCR provides greater sensitivity and allows detection and enumeration of viable or non-viable contaminants. PCR may be useful for tracking sources of microbial contaminants in the processing environment regardless of cell viability, although additional work is needed to investigate potential environmental inhibitors of the PCR.

P3-52 Evaluation of Commercial Rapid Assay Kits and a Conventional Culture Method for Detection of *Salmonella*, *Listeria*, and *Escherichia coli* O157 in Traditional Korean Foods

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Two commercial kits, VIDAS® (bioMérieux, Marcy-l'Etoile, France) and Reveal® test kits (Neogen Corp., MI, USA), for the detection of *Salmonella* spp., *Listeria* spp. or monocytogenes, and *Escherichia coli* O157 were validated by comparing the kits to the Korean Food Code Bacterial Analysis Manual (KOBAM) methods, using traditional Korean foods. Food types were inoculated with *Salmonella*, *Listeria*, and *E. coli* O157 at three different levels: a high level where all positive results were expected, as positive controls; a medium level where predominantly positive results were anticipated; and a low level where fractional recovery was predicted. For each food category and inoculum level, twenty samples were analyzed by the KOBAM, Reveal, and VIDAS® method. For each set of samples, five uninoculated controls were included. There was no statistical difference between the two commercial kits and the KOBAM methods for the three food pathogens when the inoculum levels were high (more than 100 CFU/ml or g) and medium (1– 10 CFU/ml or g). However, when the inoculum level was low (less than 1 CFU/ml or g), the sensitivity of each method was not homogeneous. It was notable that the 8-h enrichment time for Reveal 8-h kit for *E. coli* O157 was not enough to support the growth of the bacteria to detectable levels of the kit in certain Korean traditional foods such as fermented fish products. The current study showed that the inoculum level that gives fractional positives and the number of the test samples that fit for the statistical analysis were the key elements for the validation of rapid detection kits for foodborne pathogens.

P3-53 Exclusion of a False Positive Strain, *Escherichia vulneris*, from a Chromogenic Agar Plate for Specific Detection of *Enterobacter sakazakii* by Supplementing with Glucose

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Escherichia vulneris has caused false positive results on a chromogenic agar media (Druggan–Forsythe–Iversen; DFI agar, Oxoid, Hampshire, UK) that uses 5-bromo-4-chloro-3-indolyl-á, D-glucopyranoside, the chromogenic substrate of á-glucosidase. In this study, we supplemented the chromogenic agar with glucose at the ratio of 10 g per liter to eliminate the false-positives producing a modified DFI agar (mDFI). *E. sakazakii* strains showed blue-green on both DFI and mDFI agar while *E. vulneris* showed blue green colonies as false positive on DFI agar but small white colonies on mDFI agar. *E. sakazakii* and *E. vulneris* were also readily differentiated by colony color when the mixed culture of the two strains was plated on mDFI agar and incubated for 24 h. This study showed that the mDFI agar could be used for eliminating *E. vulneris* and other false positives from DFI agar by simple supplementation with glucose.

P3-54 DSC Ten-minute Assay for Detecting Staphylococcal Enterotoxin B in Apple Juice Using Piezoelectrically-excited Millimeter-sized Cantilever Sensors

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Introduction: Detection of Staphylococcal Enterotoxin B (SEB) in fruit juices and milk is conventionally done using ELISA, which uses labeled reagents, and requires about 1 h from sampling.

Purpose: The purpose is to develop a rapid assay for detecting SEB in apple juice, using PEMC sensors.

Methods: Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors were immobilized with polyclonal antibody (Ab) to SEB and tested for SEB detection in apple juice spiked with 100 femtograms and with 1 and 10 picograms of SEB in 1 mL sample.

Results: The PEMC sensor's resonant frequency decreases when target SEB binds to the surface-immobilized antibody. The total resonant frequency change obtained for the samples was 295 ± 29 (n = 2),

225 ± 15 (n = 2) and 553 ± 19 Hz, corresponding to SEB concentration of 25 fg/mL, 250 fg/mL and 2.5 pg/mL, respectively. Positive detection of the target toxin in apple juice in the sample solution was observed within 10 min. The response to control samples was negligible. Apple juice that was not spiked with SEB and PBS buffer gave responses of -10 ± 17 and -7 ± 22, respectively. Positive verification of SEB attachment was confirmed by second antibody, which resulted in a further change of 181 ± 33 Hz (n = 2). The results indicate that reliable detection of SEB at 100 femtograms/ml in 10 minutes can be achieved without sample preparation, and with label-free reagents.

Significance: The method developed in this study provides 10-min detection of a food toxin in fruit juice. The method can be extended to other toxins and other juice and milk matrices. The detection limit determined is about a factor of 10,000 times better than the conventional ELISA assay, and in a much shorter time-to-result.

P3-55 Isolation and Characterization of *Salmonella* Bacteriophages for Produce Biocontrol Applications

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Introduction: Bacteriophages are one purported means to combat bacterial pathogens on fresh produce. However, phage-mediated control of *Salmonella* spp. in sprouted seeds and on fresh-cut and whole vegetables has produced disappointing results despite high phage:host ratios. Isolating phages with better lytic activity is one option to improve the success of this approach.

Purpose: To isolate and characterise highly lytic *Salmonella*-specific phages to enhance pathogen biocontrol.

Methods: A cocktail of five *Salmonella* hosts was used in an agar overlay method to optimize phage isolation from sewage. Following plaque purification, host ranges and lytic activity were determined, using spot testing and automated turbidimetry.

Similarities between isolated phages were investigated using restriction fragment length polymorphism (RFLP) and transmission electron microscopy (TEM).

Results: Twenty-eight isolated phages, which clustered into four groups based on host range testing, demonstrated varying degrees of host-dependent lytic activity irrespective of concentration. The "Group 2" phages exerted the greatest lytic activity, but failed to infect 2 of the 8 group B isolates and all 7 of the group C1 isolates tested. Automated turbidimetry provided more useful host range and quantitative lysis data than the spot test overlay method. TEM was able to distinguish between phages on the basis of differing viral length but the RFLP analyses were inconclusive, suggesting that further optimization of the methodology is required.

Significance: Phages against bacterial pathogens such as *Salmonella* can be easily isolated and combined, making this a very flexible and inclusive approach to combat a variety of food safety issues. It is anticipated that the FDA's recent approval of *Listeria monocytogenes*-specific phages for Ready-to-Eat meat and poultry applications will generate significant interest in this approach for other Ready-to-Eat commodities such as fresh produce.

P3-56 Development of a Rapid Method for the Isolation and Detection of *Enterobacter sakazakii* and *Salmonella* spp. in Xanthan Gum

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Xanthan gum (E415) is a versatile ingredient widely used in the food industry as a thickener, lubricant, suspending agent, emulsifier and foam stabilizing agent. Xanthan gum produces a highly viscous gel and is extremely resistant to enzymatic degradation. It presents a challenging matrix for detection of bacterial pathogens such as *Salmonella* spp. and *Enterobacter sakazakii*. The food industry would benefit from a sensitive and rapid pathogen detection method, capable of analysing samples larger than the 5 g samples that are currently tested. The aim of the study was to develop a rapid and sensitive method for the detection of *Salmonella* spp. and *Enterobacter sakazakii* present at low levels (1–10 CFU/ 25 g) in larger test samples of Xanthan gum. Samples of Xanthan gum (25 g in 2.475L of BPW) were inoculated with low levels 1–10 CFU/25 g of *Salmonella* spp. and *Enterobacter sakazakii* and were pre-enriched for 7 h at 37°C. A 30-min Re-circulating Immunomagnetic Separation (RIMS) system was used to simultaneously and selectively concentrate the target bacteria from the samples. The bead bacteria complexes were then spread onto selective agar plates as follows; XLD and BGA for *Salmonella* spp.; Chromogenic agar streak plating (DFI formulation, Oxoid) and VRBGA pour plate with overlay for *Enterobacter sakazakii*. The data showed that RIMS linked to selective agar plating could detect *Salmonella* spp. and *Enterobacter sakazakii* present at low levels (1–10 CFU/25 g), and data correlated 100% with the conventional methods. The method developed is both sensitive and rapid (< 24 h). It offers significant benefits to manufacturers by allowing the rapid analysis of larger sample sizes, hence improving the safety of the product.

P3-57 Development of a Rapid Assay for the Detection of *Listeria* spp. in Environmental Swabs Using Re-circulating Immunomagnetic Separation Linked to Real-time PCR (RT-PCR)

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The rapid detection of *Listeria* spp. in environmental swabs can help facilitate the identification of problem areas in a production facility and help to improve the overall HACCP process. The aim of this study was to develop a rapid and sensitive method for the analysis of environmental sponge and/or cotton bud swabs, which allows the detection of *Listeria* spp. present at low levels (1–10 CFU/ swab) in less than 24 h. One-hundred individual foam and one hundred cotton bud swabs taken from environmental surfaces were used. Twenty of each one-hundred swab types were inoculated with *L. monocytogenes* at low levels of 1–10 CFU/swab and all swabs were pre-enriched individually in 50 ml of Half Fraser broth for 24 h at 30°C. Ten ml aliquots from the individual swabs were pooled to create a single composite sample comprised of one positive swab and four uninoculated swabs. All swab samples were also analyzed using the FDA BAM reference method. A total of 40 pooled swabs (representing 200 samples) were analysed using a re-circulating Immunomagnetic Separation (RIMS) system for 30 min to allow the capture and concentration of any *Listeria* in the samples. The recovered bead-bacteria complexes were then further analysed by RT-PCR and selective agar plates were used as detection systems. The data demonstrated a 100% correlation between all methods i.e., RIMS linked to either RT-PCR or selective agar plate and the FDA BAM method. This method is both rapid and sensitive and offers significant benefits to food producers. The method can be easily incorporated into HACCP regimens within food producing facilities.

P3-58 Rapid Isolation and Detection of *Salmonella* spp. from Chocolate Crumb, Cocoa Liquor and Cocoa Butter Using Re-circulating Immunomagnetic Separation Linked to Real-time PCR

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Chocolate crumb, cocoa liquor and cocoa butter present challenging matrices for the rapid detection of *Salmonella* spp. The chocolate industry needs a method for the detection of *Salmonella* spp. at low levels. The aim of the study was to develop a rapid and sensitive method capable of analyzing large samples for the isolation and detection of *Salmonella* spp. at levels of 1–10 CFU/ 500 g in chocolate crumb and 1–10 CFU/25 g of cocoa liquor and cocoa butter. 100 x 100 g crumb samples were used, with twenty being inoculated individually with *Salmonella* Montevideo at 1–10 CFU/sample. Enrichment in 900 ml pre-warmed (37°C) Brilliant Green Milk Broth (BGMB) was overnight. Similarly treated 50 x 25 g cocoa liquor/ butter samples were used. Re-circulating Immunomagnetic Separation (RIMS) was used to selectively concentrate *Salmonella* spp. from pooled samples, (1 x 50 ml inoculated + 4 x 50 ml uninoculated enriched samples). The bead-bacteria complexes were analyzed using RT-PCR and selective agar plates. FDA BAM *Salmonella* spp. method was used as the reference method. The data indicated that there was 100% correlation between the reference method and either RIMS linked to RT-PCR or selective agar plates at low levels (1–10 CFU/sample) for all sample types. Detection was achieved after only 16 h pre-enrichment. This method showed increased sensitivity over current PCR-based methods as well as a faster time to result (< 18 h including RT-PCR). It offers significant benefits to chocolate producers because pooling allows for larger sample sizes. The method also allows manufacturers to adopt a positive release system for their product.

P3-59 A Simple Enrichment and Lateral Flow Assay for the Rapid Detection of *Salmonella* in Poultry Environmental Samples

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Salmonella testing in poultry environmental samples is a critical step for poultry breeding stocks and hatchery products with respect to freedom from hatchery-disseminated diseases. Currently, almost all of the testing is conducted according to the conventional USDA/FSIS cultural method, which takes 4–7 days before a negative result can be reported. This is very labor intensive and time consuming. A simple lateral flow immunoassay method that followed a two-media and single-transfer enrichment protocol, i.e., RapidChek® SELECT™ *Salmonella*, was evaluated for chicken house samples, including environmental drag swabs, feed, feces, chick fluff and chick paper from at least 5 locations. These types of samples typically have very high microbial load, up to 10^9 CFU/sample. A total of 574 samples were tested by four independent laboratories as side by side comparison between the cultural method and the lateral flow method, of which 125 and 162 confirmed positives were identified, respectively. For this simple enrichment and lateral flow method, a sensitivity of 98% and specificity of 95% were demonstrated for these samples. This method was granted approval as an official method by the National Poultry Improvement Plan at its 38th Biennial meeting in 2006. Apparently, the ease of use, low cost of use and superior performance associated with this method will make it a very valuable tool for *Salmonella* testing for this market.

P3-60 A Comparative Study of Four Alternative Methods and the ISO 6579 Method for the Detection of *Salmonella* spp. in Food Products

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Salmonella is one of the most important pathogens responsible for food poisoning worldwide. Because of its low infective dose, regulations require the total absence of *Salmonella* in a defined sample size of 25 grams or larger. Detection of *Salmonella* is time consuming, often taking up to five days to

confirm a negative sample. Alternative methods using enzymatic immunoassay-based techniques or Chromogenic plates have the potential to simplify and accelerate this detection. The aim of the study was to compare the performances of four different alternative methods with the ISO 6579:2002 for the detection of *Salmonella* spp. in food products. The VIDAS Easy *Salmonella* (bioMérieux), Simple Method for *Salmonella* (SMS) (AES Chemunex), TECRA Ultima *Salmonella* (TECRA), and Transia Plate *Salmonella* Gold (Raisio Diagnostics) assays were tested according to their package insert instructions. A common pre-enrichment was performed in Buffered Peptone Water (BPW) according to incubation times and temperatures recommended by each manufacturer. A total of 175 products were tested, including both naturally and artificially contaminated samples as well as uncontaminated samples. Among the 175 samples, 100 were positive according to at least one of the methods, 98 with the reference method (98.0% sensitivity), 97 with VIDAS Easy SLM (97.0% sensitivity), and 96 with Transia Plate *Salmonella* Gold and TECRA Ultima *Salmonella* (96.0% sensitivity). Only 87 samples were positive with the SMS method (87.0% sensitivity). Except for the SMS method, statistical analysis (binomial law) showed no differences between each method and the reference method.

P3-61 Cutting Sample Preparation Time in *Salmonella* Testing with Enhanced Enrichment and Immunomagnetic Capture

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Introduction: Fast test results are crucial when screening *Salmonella* from foods. The rapid tests of today are still characterized by long sample preparation times. In order to meet the needs of the food industry, more efficient enrichment and concentration methods are needed.

Purpose: The purpose of this study was to evaluate the performance of a new 24-h MAGDA *Salmonella* method developed by Raisio Diagnostics. The method is based on enhanced 6-h enrichment after which *Salmonella* is captured with immunomagnetic particles from 10 ml of enriched sample. Plating on selective agar is used as the detection system.

Methods: Pure and serial dilutions of *Salmonella* (n = 62) and non-*Salmonella* strains (n = 39) were tested using a immunomagnetic capture method, and cultured on XLD and Rambach agar plates. Six food matrices (poultry, milk, fish, eggs, pasta bolognaise and pastries) were artificially contaminated with different levels of *Salmonella* (from 0.8 to 25.5 cells/25 g of sample) and analyzed with MAGDA and ISO 6579:2002 method. Additionally, 201 food products (spiked with stressed bacteria) were screened by using both methods.

Results: From 62 samples 58 positive *Salmonella* results were obtained (9%). Three non-*Salmonella isolates* showed typical colonies and were confirmed as *Hafnia alvei* (2) and *Proteus mirabilis* (1). The limit of detection of the method was between 1 and 3.3 cells/ml with pure strains and between 1 and 4 cells/25 g for all matrices with artificially contaminated samples. The results from different product categories showed that the MAGDA *Salmonella* method was not statistically different from the ISO method.

Significance: These data suggests that the MAGDA *Salmonella* 24 h assay provides positive results within 24 h, compared to the conventional method which requires 4 days to perform.

P3-62 New 24-hour Immunoassay Method for the Detection of *Salmonella* in Food

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Introduction: Diseases caused by *Salmonella* spp. are a significant health problem, and this pathogen has to be efficiently controlled in the food chain. Rapid monitoring methods save time and money in the food industry and are crucial for certain types of products (e.g., raw meat, ready to eat food, ultra fresh food).

Purpose: The purpose of this study was to evaluate the performance of a new *Salmonella* 24 h ELISA detection method, developed by Raisio Diagnostics and called TAG 24 *Salmonella*.

Methods: Pure and serial dilutions of *Salmonella* (n = 50) and pure non-*Salmonella* strains (n = 30) were tested with the Transia Plate *Salmonella* Gold ELISA test after enrichment in Buffered Peptone Water (18 h/37°C) and Brain Heart Infusion (4 h/41.5°C), both supplemented with the TAG24 additive. Six food matrices (poultry minced meat, raw milk, raw eggs, fish filet, pet food) were artificially contaminated with different levels of *Salmonella* strains (0, 1, 3 and 10 cells/25 g of sample) and compared with ISO 6579:2002 reference method. Food products (n = 300, containing natural samples and samples spiked with stressed bacteria), were screened by using both methods. Positive ELISA results were confirmed by streaking onto selective agar plates and biochemical identification.

Results: All the tested *Salmonella* strains were detected and no cross reactivity with other bacteria was observed. The limit of detection was assessed between 2×10^4 and 2.5×10^5 cells/ml with pure strains and between 0.3 and 1.5 CFU/25 g with artificially contaminated samples. The analysis of contaminated samples according to the 16140:2003 standard showed that the TAG24 method was not statistically different from the reference method.

Significance: These results demonstrate that the TAG24 *Salmonella* is a specific, effective, and reliable method for the detection of *Salmonella* in different food matrices within 24 h.

P3-63 An Enrichment Method for Detection and Isolation of Shiga Toxin-producing *Escherichia coli* in Cattle Feces

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Cattle are reservoirs and transient carriers of Shiga toxin-producing *Escherichia coli* (STEC). Based on fecal concentration of STEC, cattle are categorized

into super/high or low shedders (≥ 10^5 vs ≤ 10^2 CFU/g). Detection and/or isolation methods of STEC in cattle feces varied and included a wide range of enrichment media containing different antibiotics. The objective was to examine the efficacy of a new method of STEC detection in and isolation from cattle feces. To achieve that, five O157:H7 (ATCC 43888 [control non-toxin producing], 43889, 43890, 43895, and 700927) and 40 non-O157:H7 (cattle isolates belonging to the O1, O15, O26, O86, O111, O125, O127, O128, O136, O146, O153, O158, O165, O166, and O169 sero-groups) STEC isolates were tested. Briefly, all isolates were grown in tryptic soy broth (TSB) for 6 h and their concentrations were determined using their growth curves. Aliquots from each culture were immediately used for inoculating two duplicate sets of 50-mL tubes containing 1 g sterile cattle feces to simulate the high and low levels of STEC shedding (10^5 vs 10^2 CFU/g, respectively). The inoculated feces were incubated in 50 mL Brain Heart Infusion broth (for toxin induction) containing cefixime (50 µg/L), novobiocin (20 mg/L), potassium tellurite (2.5 mg/L), and vancomycin (40 mg/L) at 37°C for 12 h. The cultures were tested for toxicity using Vero (African green-monkey kidney) cells and VTEC (Verotoxin-producing *E. coli*)-Screen kit (Denka Seiken Co., Ltd., Tokyo, Japan). The STEC isolates were recovered by plating onto sorbitol-MacConkey agar, grown in TSB, confirmed as *E. coli* biochemically, serotyped, and tested for motility. Except for ATCC 43888, all isolates were detected though their toxicity and their isolation was achieved at both concentrations. Thus, our enrichment medium had no detrimental effects on growth, detection, or isolation of this wide range of STEC isolates.

P3-64 DSC Optimization of the Enrichment Protocol for Detection of *Escherichia coli* O157:H7 in Ground Beef by the Vidas *E. coli* O157:H7 (ECO) Screening Test

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Introduction: *Escherichia coli* O157:H7 is a foodborne pathogen that has become a public health priority. Ground beef has been implicated as the most common food source for infection. Different rapid methods are currently used for detecting this foodborne pathogen in ground beef, and these methods vary in test sample size, enrichment protocols and detection methodology. There is a need for evaluating and comparing rapid, simple and cost effective sampling and testing protocols to improve *E. coli* O157:H7 detection.

Purpose: The aim of this study was to provide optimal sampling and enrichment protocols for the rapid and accurate detection of *E. coli* O157:H7 in ground beef, using the AOAC RI validated VIDAS ECO test.

Methods: Three different one-step enrichment protocols in Buffered Peptone Water (BPW) were compared at incubation temperatures of 37°C and 41.5°C. "Sample-to-enrichment" broth ratios included 1/10 (25 g of ground beef in 225 ml BPW), 1/3 (75 g ground beef in 175 ml BPW) and 1/2 (125 g of ground beef in 125 ml BPW). Performance of each sampling and enrichment protocol was assessed based on the growth ratio of the background microflora versus target *E. coli* O157:H7 cells and by detection of *E. coli* O157:H7 using the VIDAS ECO test.

Results: This study demonstrated that the detection of *E. coli* O157:H7 was significantly better at the sampling enrichment ratios of 1/10 and 1/3 combined with a temperature of 41.5°C. Additional experiments with stressed *E. coli* O157:H7 strains suggested two protocols, 25 g ground beef sample in 225 ml of enrichment in BPW for 8 h at 41.5°C and 75 g ground beef sample in 175ml of enrichment BPW for 18 h at 41.5°C, as being optimal.

Significance: The new simplified protocols offer different benefits to the beef industry: results within 9 or 18 h, cost effectiveness by using pooling, and accuracy and simplification of the work-flow.

P3-65 New Method for Simultaneous Detection of *Listeria* Species and *Listeria monocytogenes*

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The objective of this study was to evaluate a new immunoassay for simultaneous detection and differentiation of *Listeria* species and *Listeria monocytogenes* in food samples, the VIDAS® *Listeria* DUO (LDUO). The test is based on an automated Enzyme Linked Fluorescent Assay (ELFA) targeting specific virulence and flagellar proteins. Samples are culturally enriched for a total of 44–48 h in LX broth, before testing in the VIDAS® instrument. Positive results are then confirmed by streaking enrichment broths onto selective agar. The study was designed to provide pre-collaborative data as part of the AOAC approval process. Inclusivity and exclusivity were both 100% using a 145 *Listeria* and 54 non-*Listeria* species. Lot-to-lot testing showed very good reproducibility (CVs below 15%) and the inter assay and inter instrument reproducibility were excellent (CVs below 7%). The inoculated food study includes 15 foods (25 replicates of each food) compared to relevant reference methods: AOAC method 993.12 for dairy products, USDA FSIS method for meat and poultry, and FDA BAM method for vegetables and fish. The LDUO correlates well with cultural methods and has the advantages of convenience and reduced time for a result. Routinely testing for both *Listeria* spp. and *L. monocytogenes* can provide food manufacturers with the relevant information to reduce the risk of product recalls and optimize corrective actions.

P3-66 Evaluation of the Effect of Various pH Levels on 3M™ Petrifilm™ Aerobic Count Plates, 3M™ Petrifilm™ *E. coli* Coliform Count Plates, and 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates on Microbial Growth When Compared to the Reference Method

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Current 3M™ Petrifilm™ Plate method may require an adjustment of the diluted sample to pH 6.6–7.2 (AOAC 990.12, 991.14, 996.02, and 2003.01). When a wide variety of products are tested with varying pH, the measurement and adjustment of pH of every sample can be time consuming. Sixty food samples were tested, three samples from each of the following 13 food types: raw ground beef, luncheon meat, ham, raw marinated chicken, raw turkey, cooked beef, soup, dessert item, frozen vegetables, fresh vegetables, fruit, flours, and dog food. Food samples were individually inoculated with low, medium and high levels of a cocktail of *Escherichia coli*, *Enterobacter aerogenes*, and *Klebsiella oxytoca* and then adjusted to pH 5.7 +/- 0.1 and pH 7.9 +/- 0.1. Petrifilm Aerobic, *E. coli*/coliform, and *Enterobacteriaceae* Count Plate results were compared to the agar or MPN reference method. There was no statistical difference (μ >0.05) between the test results for the reference method and the Petrifilm Aerobic Count Plate for raw ground beef, luncheon meat, cooked beef, ham, dessert items, flour, and wet dog food when the pH of the samples was adjusted to 5.7 and 7.9. This was also true for the *E. coli* coliform Count Plate with luncheon meats, cooked beef, ham, raw turkey, marinated raw chicken, dessert items, and frozen vegetables. For the Petrifilm *Enterobacteriaceae* count plate, when the pH of the sample was adjusted to 5.7 and 7.9, there was no significant difference (μ >0.05) between the results for it and the reference method for cooked beef, ham, dessert items, flour, soup, and wet dog food. With the 3M™ Petrifilm™ performing as well as the reference method with sample pH of 5.7 and 7.9, the acceptable range of pH for the methods can be widened, leading to valuable time savings in the laboratory as fewer foods require pH adjustment.

P3-67 Preliminary Evaluation of Reduced Enrichment Media Volumes for the Detection of *Escherichia coli* O157:H7 from Beef Trimmings in Conjunction with a Rapid Immunological Screen

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Recent research has reported the ability to reduce the amount of enrichment media used when enriching ground beef and beef trimmings for the detection of *Escherichia coli* O157:H7. However, the research only discusses the enrichment process when the final detection method is direct plating onto growth agar or immunomagnetic separation with plating onto Chromogenic agar. The research does not discuss the effect of a decrease in enrichment media when the detection step involves the use of immunoassays or molecular detection systems. The research also reports inoculation levels of approximately 2 organisms/gram. The objective of this study was to validate the use of reduced volumes of enrichment media for the detection of *Escherichia coli* O157:H7 in beef trimmings. The beef trimmings (n = 25) were inoculated with composite cultures (0.023–0.024 CFU/gram) prepared from 3–5 strains of *Escherichia coli* O157:H7 from the Silliker Food Science Center Culture Collection (FSC–CC). They were then stored for 48 h at 4°C. The inoculated beef trimmings were tested for *E. coli* O157:H7 using the standard protocol for Strategic Diagnostic Inc.'s RapidChek® *E. coli* O157:H7. Tryptic soy broth and SDI media were tested at ratios of 1:10, 3.75:10 and 1:3 weight to volume. Data indicated there was a significant difference at the 1:10 dilution level (x2 of 10.08) but there was no significant difference in recovery at the other two dilution levels. This experiment suggests that lower dilutions and TSB are viable alternatives to a 1:10 even with rapid screening. More work is needed to further explore this option with a larger sample of rapid screening methods.

P3-68 Detection of *Escherichia coli* O157:H7 in Artificially Contaminated Spinach by Pathatrix™ Immunomagnetic-capture, Real-time PCR and Cultural Methods

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Escherichia coli O157:H7 has been responsible for a number of foodborne disease outbreaks associated with fresh produce. A recent outbreak of *E. coli* O157:H7 illness associated with packaged baby spinach resulted in 200 confirmed illnesses, with 98 hospitalized and 3 deaths. This warranted the evaluation of rapid methods such as selective enrichment with Modified Buffered Peptone water (mBPW) combined with Immunomagnetic Separation (IMS), real-time PCR, detection and isolation on several selective agars. This approach was compared to the lengthier FDA BAM enrichment (EB) and isolation procedure for the detection of *E. coli* O157:H7 in spinach. Spinach samples were inoculated with low (0.1 CFU/g) and high (1 CFU/g) levels of *E. coli* O157:H7 ATCC 35150. Rinsates were prepared from spinach samples and mixed with mBPW and EB. The mBPW enrichment was resuscitated 5 h at 37°C followed by addition of selective agents and overnight enrichment at 42°C. DNA templates were prepared from 1 ml portions of the mBPW and EB enrichments. In addition the *E. coli* O157:H7 was concentrated from the entire volume (250 ml) of mBPW by IMS using the Pathatrix™ system. Templates were assayed using multiplex real-time PCR detection of *stx*1, *stx*2, and a unique *E. coli* O157:H7 uidA gene target. The mBPW

enrichment and the immunomagnetic beads were cultured on Levine's EMB, Tellurite-Cefixime Sorbitol MacConkey (TCSMAC), and R & F® Chromogenic O157agar for isolation. The mBPW enrichment method was found to provide 100% sensitivity in detecting and identifying *E. coli* O157:H7 at 0.1 CFU/g in artificially contaminated spinach. Variation in performance of the selective agars was noted. TCSMAC and R & F® agars performed very well, while Levine's EMB showed less recovery due to lower selectivity and the presence of interfering background organisms on the plates. The FDA BAM method provided equivalent sensitivity but required additional time to complete.

P3-69 Detection of *Escherichia coli* O157:H7 in Food Using Real-time Multiplex PCR Assays

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Introduction: *E. coli* O157:H7 is an important foodborne pathogen, and foods of bovine origin and fresh produce have been linked to outbreaks. Sensitive and rapid methods for detection of the pathogen are needed.

Purpose: The purpose of this study was to develop real-time multiplex PCR assays that can be used to detect *E. coli* O157:H7 in different foods.

Methods: Samples of apple cider, raw milk (25 ml), ground beef, and lettuce (25 g) were inoculated with 2 or 20 CFU of *E. coli* O157:H7 strain 380–94 in 225 ml of Rapid-Check *E. coli* O157:H7 Enrichment Broth and incubated at 42°C at 100 rpm. After 8 and 20 h, aliquots of the enrichments were removed, and DNA extraction was performed. Multiplex PCR assays using TaqMan probes and primers targeting the *stx*1, *stx*2, and wzyO157 genes in combination with probes and primers targeting either the fliCh7 or the *eae* genes were performed using Omnimix tablets and the Smart Cycler.

Results: The sensitivity of the real-time multiplex PCR assay was 500 CFU/PCR. *E. coli* O157:H7 was detected in food samples inoculated with both 2 and 20 CFU/ml or g after both 8 and 20 h of enrichment. Enrichments of uninoculated food samples were negative using the multiplex PCR targeting the *stx*1, *stx*2, wzyO157, and *eae* genes; however, using the assay targeting the *stx*1, *stx*2, wzyO157, and fliCh7 gene combination, a positive result was always obtained for the fliCh7 gene using ground beef enrichments. Other primer sets targeting the fliCh7 gene gave similar results; therefore, the fliCh7 gene may not be a suitable target for detection of *E. coli* O157:H7 in ground beef.

Significance: The real-time multiplex PCR assay targeting the *stx*1, *stx*2, wzyO157, and eae genes is sensitive and specific and can be used for detection of *E. coli* O157:H7 in apple cider, raw milk, lettuce, and ground beef.

P3-70 Evaluation of Pathatrix (Immunomagnetic Concentration) and BAX (PCR) Using Two Nonselective Enrichment Broth for the 24-hour Recovery of Stressed *Listeria* Species from Artificially Contaminated Sponges

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The detection of *Listeria* species in the environment is of great concern to many food manufacturers. Current screening methods for *Listeria* take anywhere from 24 to 48 h to screen samples. Many systems use proprietary media coupled with an end point detection system such as PCR for rapid screening. The purpose of this study was to determine the efficacy of 2 non-selective broths, Universal Preenrichment Broth (UPB) and Buffered Peptone Water (BPW) enrichment broth, coupled with immunomagnetic concentration, to screen samples for *Listeria* species in 24 h. Sponges (n = 25) were inoculated with a 5-strain cocktail of stressed *Listeria* at a level of 3.76 organisms per sponge. Each sponge was incubated for 24 h at 30°C in 100 milliliters of BPW and UPB respectively. After incubation, 50 ml aliquots were removed and pooled for immunoconcetration and then tested by PCR. Enrichments were pooled in combinations 0 of 5 positive sponges, to 5 of 5 positive sponges. Data indicates there was no significant difference in detection efficiency between the 2 broths. However, data also indicates there was a statistical significant difference $t = 5.42$ ($a = 0.05$) between broths when comparing the level of organisms recovered at 24 h. From this feasibility study we determined that both broths show promise as nonselective enrichments when combined with immunoconcentration and PCR.

P3-71 Efficiency of Transport Media for Recovery of *Listeria* from Milk Biofilm and Meat Processing Plant Environmental Swabs

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Introduction: Environmental samples are useful in control monitoring of *Listeria* in the production plant. Determining the efficacy of the medium used for transporting the swabs to the laboratory is vital to ensure validity of the monitoring.

Purpose: The objective was to evaluate three swab transport media for recovery of *Listeria* from a model milk biofilm after sanitization and from environmental swabs obtained from a meat processing plant after storage at 4° or 25°C for 24 or 48 h. The transport media consisted of a proprietary formulation (PF), D/E Neutralizing Broth (DE), and Neutralizing Buffer (NB).

Methods: Sponge swabs were rehydrated in PF, DE or NB. *Listeria* model milk biofilms on stainless steel were swabbed after rinsing and treated with a quat sanitizer. The transport media expressed from the swabs were plated to determine recovery efficiency. Fifty-four meat processing plant swabs were cut into thirds, and each third was immersed in

PF, DE or NB, and incubated at 4°C for 24 h; 4°C, for 48 h; or 4°C for 24 h followed by 25°C, for another 24 h. Samples were analyzed according to USDA detection methods.

Results: *Listeria* recovery from the sanitized milk biofilm, measured by reduction in log count from initial level, was 2.7 ± 0.6, 3.0 ± 0.7 and 3.3 ± 0.3 by DE, PF and NB, respectively. For the meat processing environment, PF, DE and NB performed similarly if swabs were stored at 4°C for 24 h (57, 57 and 56% positive, respectively). For 48 h storage at 4°C, efficiency was 54, 48 and 43%, respectively. For 24 h at 4°C followed by 24 h at 25°C, PF and DE produced 52% positives, whereas NB recovered only 41%.

Significance: Transport media may vary in efficiency for recovering *Listeria* from environmental swabs and should be evaluated for optimal use in control monitoring programs.

P3-72 Evaluation of 3M™ Petrifilm™ Staph Express Count System for *Staphylococcus* spp. Detection in Individual Cow Milk Samples

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Mastitis is one of the principal causes of economic loss and changes in milk quality. *Staphylococcus* spp. is considered a major contagious pathogen of this disease and its presence in herds means decreased milk production and economic drawbacks. The objective of this study was to evaluate the efficacy of the 3M™ Petrifilm™ Staph Express Count System for detecting *Staphylococcus* spp. in individual cow milk samples compared to traditional methodologies using Baird Parker Agar (BPA) and Blood Agar (BA). The study was carried out in an experimental farm located in Minas Gerais State, Brazil. Fifty-five milk samples were collected from composite quarters of cows after foremilking and teat disinfection using 70% alcohol. Detection of *Staphylococcus* spp. using 3M™ Petrifilm™ Staph Express Count Plates was compared to traditional methodologies using BPA and BA. The results were analyzed using a chi square test. In 24 h, 3M™ Petrifilm™ Staph Express Plates detected *Staphylococcus* spp. in 53 samples tested 27 out of 53 (50.9%), *S. aureus* confirmed. The agar methods were only able to detect *Staphylococcus* spp. (49 using BPA and 12 using BA). 3M™ Petrifilm™ Staph Express Plates were more efficient ($P < 0.005$) than BPA and BA in the detection of infections of cows by *Staphylococcus* spp. It can be concluded that 3M™ Petrifilm™ Staph Express Count Plates can be an important tool to quickly identify cows infected by *Staphylococcus* spp. and *S. aureus* and thereby aid milk producers in improvements to control mastitis caused by this pathogen.

P3-73 ValidCheck™– A Rapid and Accurate Detection System for *Staphylococcus aureus* in a Variety of Foods

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Introduction: Foodborne bacterial contamination continues to be a foremost public health concern. Early detection and intervention is critical not only to ensure cost-effective production, but also to provide safe food to consumers. ValidCheck™ is designed to detect fluorescence emitted from a target foodborne pathogen using fluorescent-immunomagnetic reagents and a Roto-Spectrofluorometer.

Purpose: The purpose of this study was to evaluate the performance of the ValidCheck™ system for the detection of *S. aureus* in a variety of foods.

Methods: *S. aureus*-negative samples (non-fat dry milk, ham, salami, hot dogs, sausage, bacon, chicken salad, and potato salad) were inoculated with *S. aureus* (ATCC 12600U) at 0 cells/10 g and 10 cells/10 g (five replicates of each sample). These samples were enriched using modified *Staphylococcus* broth (1:25) and incubated at 37°C for 23 h. One milliliter of each sample was taken post-enrichment, incubated with *S. aureus* specific reagents, and measured via the Roto-Spectrofluorometer.

Results: Results indicate that the ValidCheck™ system was able to detect all 40 spiked samples (detection limit of 1 cell/ g). All 40 non-spiked samples were found to be negative. Results were confirmed by FDA Bacteriological Analytical Manual (BAM) methods. Results also agreed with the reference culture method ($x^2 < 3.84$). Specificity was evaluated using pure cultures of *L. monocytogenes*, (ATCC 19115), *C. glutamicum* (ATCC 14020), *E. coli* O157:H7 (ATCC 700378) and S. Enteriditis (ATCC 49216). These non-target organisms were found negative by the ValidCheck™ system when *S. aureus*-specific reagents were used.

Significance: This study demonstrates the capability of the ValidCheck™ system to detect *S. aureus* at the level of 1 cell/ g in a variety of foods within 24 h.

P3-74 Highly Specific and Sensitive Detection and Quantification of *Staphylococcus aureus* Using Real-time PCR

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Introduction: *Staphylococcus aureus* is a gram-positive pathogen that can contaminate a variety of foods. *S. aureus* is found in meat, poultry, egg, milk and dairy products, salads such as egg and tuna, and baked goods with creme fillings. Specific and sensitive detection of *S. aureus* has been achieved using an assay based on careful target selection and bioinformatics algorithms.

Purpose: The aim of this study was to develop a specific, reliable, and simple-to-use Real-time PCR assay for *S. aureus* and demonstrate its utility for rapid and sensitive screening of enrichments of meat and dairy products.

Methods: A duplex Real-Time PCR assay was developed against the RNaseP gene of *S. aureus* and a positive control sequence to monitor PCR inhibition. Assay performance was tested on an inclusivity panel of *S. aureus* isolates and an exclusivity panel of related *Staphylococcus* species (including *S. epidermidis*, *S. schleiferi*, *S. carnosus*). Sensitivity of the assay was evaluated using a quantified *S. aureus* DNA sample.

Results: The *S. aureus* assay, tested in lyophilized and tabletted form, detected 25 out of 25 (100%) *S. aureus* isolates in the inclusivity panel and none of the 27 (0%) species in the exclusivity panel. Limit of detection was estimated at 10 genomic copies of *S. aureus*. In spiked and naturally contaminated ground beef, *S. aureus* levels of 10 CFU per gram were detected within 24 h.

Significance: This study demonstrated that the RNaseP gene in *S. aureus* is unique and enables accurate identification of *S. aureus* from other *Staphylococcus* species. This specificity combined with the high sensitivity of the assay and ease-of-use would be amenable to *S. aureus* screening of diverse foods.

P3-75 TEMPO® EC (*E. coli*) Method for the Enumeration of *Escherichia coli* in a Variety of Foods: AOAC Research Institute Independent Laboratory Study

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Introduction: The TEMPO® EC (*E. coli*) method is an automated system for the enumeration of *E. coli* in foods. The TEMPO system utilizes an enumeration card, which contains selective dehydrated culture media, and computerized filling and reading stations for the automated determination of the Most Probable Number (MPN).

Objective: As part of the AOAC Research Institute (RI) validation process, an independent lab study was conducted to compare the analytical results of the Tempo® EC system to the AOAC Official Methods 966.23 and 966.24 for the detection of *E. coli* from raw ground beef, raw ground chicken, ice cream and raw green beans.

Methods: Three levels of inoculation were performed for each matrix (50 CFU/g, 500 CFU/g and 5,000 CFU/g), except for raw ground chicken, which was naturally contaminated. For the Tempo method, five 25 g replicate portions of raw ground beef; raw ground chicken and raw green beans were added to 225 ml of Butterfield's dilution buffer and were stomached for 2 min. For ice cream, 11 g was added to 99 ml of Butterfield's buffer and stomached. The samples were incubated at 35 ± 1°C for 24 h, then analyzed by the Tempo reader. Each Butterfield's buffer diluted TEMPO sample was confirmed for *E. coli* in LST broth. The comparative MPN reference method analysis was performed according to the AOAC method 966.23 with confirmation of *E. coli* by the AOAC method 966.24. Regression analyses, difference log distributions and t-tests at the 5% level were used to analyze the data and compare performances.

Results: The TEMPO method was shown to be statistically equivalent to the reference method in both the internal and independent food studies.

Significance: Overall results indicate that the TEMPO EC method is an acceptable alternative method for the enumeration of *E. coli* in foods.

P3-76 Evaluation of the VIDAS Staph Enterotoxin II (SET 2) Immunoassay Method for the Detection of Staphylococcal Enterotoxins in Foods: Collaborative Study

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A multilaboratory study was conducted to demonstrate that the VIDAS SET 2 method can detect staphylococcal enterotoxin (SET) in foods. Five food types (cooked chicken, ham, potato salad, pasteurized liquid, whole milk and canned mushooms) were spiked, each with a different enterotoxin (A, B, C1, D or E), and tested at 0.25 ng/g and 0.5 ng/g SET levels. Uninoculated controls were also included. A total of 19 laboratories representing government and industry participated. In this study 1,674 test portions were analyzed, of which 1,638 were used in the statistical analysis. Of the 1,638 test portions used in the statistical analysis, 1,104 were inoculated test portions, of which 1,073 were positive by the SET 2 method. The overall detection rates were: cooked chicken–97.8%, ham–98.5%, potato salad–99.5%, liquid whole milk–89.8% and canned mushooms–100%. The high detection rate indicates that the VIDAS SET 2 method is capable of detecting staphylococcal enterotoxins in foods at 0.25 ng/g.

P3-77 Evaluation of the TEMPO EC Test for Quantification of *Escherichia coli* from the Korean High-salt Seasoning

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Introduction: Rapid and accurate detection and quantification of *E. coli*, as one of the important indicator organisms in food industry to assess the quality and safety of foods and food ingredients, is very important. The TEMPO® system has been developed to replace serial dilutions, media preparation and tedious plate reading with an automated enumeration based on MPN method. The Korean high-salt seasoning containing about 40% salt widely used to promote the flavors of Korean traditional foods is chosen as a matrix.

Purpose: The purpose of this study is to evaluate *E. coli* detection capability of TEMPO® EC for Korean high-salt seasoning in comparison with the enumeration method authorized by FDA.

Methods: GUD-positive *E. coli* isolates (ATCC 25922) were inoculated into Korean high-salt seasoning and wheat flour containing salt gradient (0, 10, 20, 30, 40 and 50%). *E. coli* enumeration was performed by both TEMPO® and MPN presumptive test with LST-MUG authorized by FDA.

Results: For the Korean high-salt seasoning, the sample interference in the detection of *E. coli* was found, when samples were diluted into 1/40 and 1/400. In the case of wheat flour with salt gradient (0%~50%),

which did not contain any inhibitors other than salt, the results showed that high concentration of salt in food matrix did not inhibit the detection capability of TEMPO®. With further decimal dilutions (1/400, 1/4000 and 1/40000) to diminish the sample interference in the Korean seasoning matrix, TEMPO® EC test gave equivalent results to those from MPN presumptive test authorized by FDA.

Significance: This study suggested that, with decimal dilutions, the automated TEMPO® system could give rapid and accurate results in detection of *E. coli* in the Korean high-salt seasoning matrix.

P3-78 A Study to Evaluate Direct Plating onto Chromocult® Coliform Agar for the Enumeration of Fecal Coliforms and *Escherichia coli* in Raw Shellfish

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Introduction: Raw shellfish are routinely tested for fecal coliforms (FC) and generic *Escherichia coli* (EC). The internationally accepted method for shellfish testing for FC and EC uses a five-tube multiple dilution fermentation series providing the Most Probable Number (MPN). Alternative methods can be faster, more accurate and less costly but have not been validated on raw shellfish compared with the MPN method.

Purpose: The purpose of this study was to compare two methods for FC and EC enumerations in raw, naturally contaminated clams, mussels and oysters—the MPN standard method and direct plating onto Chromocult® Coliform Agar (CCA).

Methods: Shellfish homogenate from 680 sub-samples were used to inoculate the CCA plates; 0.5 mL of the homogenate was spread onto each of 8 plates per sub-sample and incubated at 44.5°C for 24 h. Dark blue to violet colonies and/or salmon to red colonies were counted and recorded. Results were then compared to the standard MPN method results.

Results: Oysters did not exhibit typical growth on CCA and were excluded from the statistical analyses. There was excellent agreement of the distribution of sub-samples based on the compliance levels between the CCA counts and the MPN method counts for both FC and EC. Comparison of individual sub-samples between the CCA counts and a range of the MPN method counts showed excellent agreement for EC (95–97%). The agreement for FC was 69–80% where FC counts in clams tended to have a greater value than the MPN method counts.

Significance: The direct plating method using Chromocult® Coliform Agar is an acceptable alternative to the MPN method for the enumeration of *E. coli* in raw mussels and clams.

P3-79 Comparison of Compact Dry Yeast and Mold, Petrifilm Yeast and Mold, and Conventional Agar Media for Enumerating Yeasts and Molds in Foods

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A study was done to compare Nissui Compact Dry Yeast and Mold (CDYM) medium, 3M™ Petrifilm™ Yeast and Mold (PYM) Count Plates, Dichloran Rose Bengal Chloramphenicol (DRBC) agar and Dichloran 18% Glycerol (DG18) agar, for enumerating yeasts and molds naturally occurring in 97 foods (grains, beans, vegetables, fruits, nuts, dairy products, meats, and miscellaneous processed foods and mixes). Correlation coefficients were: DG18 vs DRBC (0.93), PYM vs DRBC (0.81), CDYM vs DG18 (0.81), PYM vs DG18 (0.80), CDYM vs DRBC (0.79), and CDYM vs PYM (0.75). The number of yeasts and molds recovered from a group of foods (n = 32) analyzed on a weight basis (CFU/g) was not significantly different ($P > 0.05$) when samples were plated on DRBC, DG18, PYM, and CDYM. However, the order of recovery from foods (n = 65) in a group analyzed on a unit or piece basis, or a composite of both groups (n = 97), was DRBC > DG18 = CDYM > PYM. Compared to PYM, CDYM recovered equivalent, significantly higher ($P \leq 0.05$), or significantly lower numbers of yeasts and molds in 51.5, 27.8, and 20.6%, respectively, of the 97 foods tested; respective values were 68.8, 15.6, and 15.6% in the small group (n = 32) and 43.1, 33.8 and 23.1% in the large group (n = 65). The two groups contained different types of foods, the latter consisting largely (73.8%) of raw fruits (n = 16) and vegetables (n = 32). Differences in efficacy of the four methods in recovering yeasts and molds from foods in the two groups are attributed in part to differences in genera and predominant mycoflora in foods comprising the two groups. While DG18, CDYM, and PYM appear to be acceptable for enumerating yeasts and molds, overall, DRBC recovered higher numbers from the 97 test foods.

P3-80 Application and Evaluation of the TEMPO® System as a Fast Method for the Quantification of Microorganisms in Food

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Introduction: In recent years, the application of rapid microbiological methods as alternatives to traditional cultural methods has become increasingly important in the food industry. The currently available rapid methods mostly allow for the qualitative detection of foodborne pathogens and are predominantly based on immunological or molecular biology techniques. However, the TEMPO® system (bioMérieux), which is based on the most probable number method, represents a rapid approach for the quantitative assessment of microorganisms in food.

Purpose: The aim of this study was to evaluate the performance of the TEMPO® system for the enumera-

tion of the total viable count (TVC), total coliforms (TC) and *Escherichia coli* (EC) in comparison to a cultural method.

Methods: The TEMPO® system was used to determine TVC, TC and EC quantitatively for 285 samples from meat (n = 56), meat products (n = 56), raw milk (n = 51), dairy products (n = 37), carcasses (n = 40) and chocolate (n = 45) in various dilutions (1:40; 1:400; 1:4000) according to manufacturer instructions. For the cultural method, ascending dilutions from 1:10 to 1:1,000,000 were plated on PC (30°C; 72 h), rapid *E. coli* (37°C; 24 h) and VRBL agar (30°C; 24 h).

Results: For TVC, the correlation coefficients between the methods were 0.66, 0.68, 0.73, 0.77, 0.84, and 0.84 for dairy products, chocolate, raw milk, carcasses, meat and meat products, respectively. Repeated testing (TEMPO® system) of meat and meat-product samples revealed that results varied by less than 0.4 log units (0.11–0.32 log units). However, 27 of the 285 samples could not be evaulated as they were beyond the detection limits of the TEMPO® system. In the evaluated samples, TC and EC were not found with either method.

Significance: The TEMPO® system is a fast and convenient technique for the quantification of microorganisms in food. Repeated testing yielded reproducible TVC results. However, results obtained by the TEMPO® system were influenced by the food matrix.

P3-81 Results of a MicroVal EN ISO 16140 Validation of the Compact Dry Total Count Plate Method for the Enumeration of Total Viable Microorganisms in Foods

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Introduction: Compact Dry Total Count Plates (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 centre of the self-diffusible medium. The Compact Dry Total Count (TC) method contains the redox indicator tetrazolium salt and is an alternative method to the standard plate count, enabling determination of aerobic colony counts in foods after 48 h incubation. This study presents the results of a MicroVal validation in accordance with the principles of method validation protocol EN ISO 16140.

Purpose: Comparison of the Compact Dry TC method against a standard method (ISO 4833; 2003) using validation protocol EN ISO 16140.

Methods: The Compact Dry TC method (30°C/48 h) was performed in accordance with the manufacturers instructions and ISO 4833 was a 1 ml pour plate (30°C/72 h). The method comparison study compared both methods for the enumeration of total aerobic microorganisms in naturally and artificially contaminated raw ground beef, cooked chicken, lettuce, milk powder and frozen fish and the interlaboratory study involved 13 laboratories each testing artificially contaminated milk samples by both methods. Results were analysed using the principles of EN ISO 16140 and calculations/data transformations were performed in Minitab statistical analysis software (v14, Minitab Inc).

Results: The method comparison results clearly showed Compact Dry TC method to be equivalent to the reference method for a range of foods (calculated correlation coefficient R_2 = 97.7%). There appeared to be no significant difference between the results obtained with the method after 48 h and 72 h (R_2 = 99.8%). Interlaboratory study results revealed no evidence of differences in reproducibility between the methods.

Significance: No substantial differences were found between the Compact Dry TC plate method and the reference method ISO 4833 (2003) for the enumeration of total viable microorganisms at 30°C.

P3-82 A Comparative Evaluation of the TEMPO® TVC Method for the Enumeration of Viable Aerobic Mesophilic Flora in Foods

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Introduction: The TEMPO® TVC (Total Viable Count) method was developed for the automated enumeration of total aerobic mesophilic flora in foods. The method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number.

Objective: As part of the AOAC Research Institute validation process, the TEMPO TVC method was compared to the Standard Methods for the Examination of Dairy Products (SMEDP) for dairy products and AOAC Official Method 966.23 for all other foods.

Methods: Eighteen naturally 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, 0.1 ml of diluted food sample was added to a TEMPO medium vial that had been reconstituted with 3.9 ml of sterile distilled water. The inoculated medium in the TEMPO vial was then transferred and sealed into the TVC card by the automated TEMPO filler. The inoculated TEMPO TVC cards were incubated for 40–48 h at 35 ± 1°C or 32 ± 1°C for dairy products. Cards were read using the automated TEMPO reader. Standard method testing was performed as detailed in the SMEDP for dairy products and in the AOAC Official Method for all other foods.

Results: For the majority of samples tested, there was no significant difference, for the mean log counts or for repeatability, between the TEMPO TVC method and the standard method using a paired t-test and f-test at the 5% level.

Significance: TEMPO TVC provides an automated, accurate method for the enumeration of total aerobic mesophilic flora in foods.

P3-83 A New Approach for Workflow Evaluation in Food Microbiology Laboratories: Automated MPN Method Versus Plate Count Method

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While several rapid microbiological test methods have addressed the need to detect pathogens quickly and with a degree of automation, the development of rapid and automated methods directed at the enumeration of quality indicator microorganisms have been few in number. The TEMPO® system is an innovative automated method associating an enumerating card in association with a medium specifically adapted to ensure a rapid enumeration of microorganisms. This method was developed to replace preparation of plates, inoculation, counting of plates and confirmation by using an automated Most Probable Number (MPN) technique to enumerate bacteria. The purpose of this study was to develop a method to evaluate and compare the time needed to analyze a series of twenty-five food samples, three tests per sample (Total Viable Count, Total Coliforms and *Escherichia coli*) by the traditional plate count methods and by the automated method. The traditional methods were PCA 72 h at 30°C for the enumeration of total viable count, Rapid *E. coli* at 37°C for 24 h for the enumeration of Total Coliforms and TBX at 44°C for 24 h for the enumeration of *E. coli*. The study was performed in an independent external laboratory with a very efficient workflow and optimized ergonomics. Clocking was done to quantify the time needed for each step on both methods: media preparation, sample preparation, test preparation, incubation, reading and reporting. A total of 232 min was necessary to perform the 75 tests with the plate count methods, compared to 106 min with the TEMPO method, 3.1 min and 1.2 min per test respectively. Reading and reporting that account for 35% of the test time for traditional methods was reduced by 83% with the TEMPO method. Globally, the TEMPO method offers sixty percent economic savings in terms of labor.

P3-84 Anti-counterfeit Systems Food Research, Modeling and Applications: Perception, Risk Assessment and Mitigation

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Introduction: Product counterfeiting and diversion in certain industries, such as food, pharmaceuticals, automotive parts, chemicals, can have deadly consequences, in addition to by-passing regulatory and safety governance structures, undermining the intellectual property and profits of legitimate enterprises, reducing tariffs and taxes paid, and reducing confidence in institutional and governance systems. Globalization has facilitated an (under) estimated trade of $300 billion in illicit goods and underground markets. The increasing global manufacture and transport of goods creates significant opportunities for counterfeiting. Related intellectual property losses cover a broad range of creative and industrial endeavors—processes, materials, packaging.

Purpose: This session will examine consumer and business risk perception and risk assessment with respect to counterfeit products and present a framework to identify the scope and risk of these products across industrial sectors. It will also highlight the actuarial perspective of the insurance industry regarding risks of counterfeit products.

Methods: A scholarly review of counterfeit food threats was conducted and correlated with expert interviews. Risk mitigation steps were reviewed and recommended.

Results: A business case for additional anti-counterfeit food research was validated.

Significance: Examples such as HACCP demonstrate the food industry's diligence for risk assessment and process improvement, but the counterfeit food theat is underestimated.

P4-01 An Evaluation of Ozonated Water as an Alternative to Chemical Cleaning and Sanitization of Beer Lines

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A reduction in beer quality may arise from biofilm development in beer lines and regular cleaning is therefore essential. Ozonated water was investigated for use as a cleaning and sanitization agent for commercial beer lines as it leaves no residues, preventing possible product taint. A comparison between traditional chemical beer line cleaner and a novel ozonated water system was made using a beer line model. The model system was challenged with organisms isolated from a brewery (*Enterobacter* spp. and yeast). The effect of each treatment on survival of the organisms was determined and formation of biofilm was investigated. Four sites in the model system were investigated for biofilm development and control. Non-recirculation of media though the system (i.e., single pass of sterile medium though all parts of the system) mimicked the effect of biofilm formation upon the microbial load of dispensed beer. Both cleaning systems were effective at reducing biofilm from initial levels (6–7 log CFU/cm^3) by a minimum of 2.76 log (ozone) and 1.71 log (chemical). Ozonated water resulted in a statistically significantly lower number of recovered microbes than the chemical cleaner for intake and tubing samples but not the fob detector or media. Cleaning with ozonated water resulted in significantly lower viable cell counts in model dispensed beer. In repeated cleaning and regrowth cycles, the ozone achieved significantly greater log reductions than the chemical cleaner. This effect was less apparent on the third and fourth cycles of cleaning and regrowth. The findings were supported by the direct observation of the biofilm using epi-fluorescent microscopy. This technique showed that the ozone seemed to remove large portions of the biofilm from the glass slide whereas the chemical cleaner merely killed a proportion of the cells. The study concludes that ozonated water has the potential for use as a beer-line sanitizer.

P4-02 Withdrawn

P4-03 DSC Development of Novel Isolation Media for Guaiacol-producing *Alicyclobacillus* spp.

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The role of *Alicyclobacillus* spp. on the spoilage of pasteurized fruit juices has been reported since 1982. Our previous research indicated that the ability to produce guaiacol and cause spoilage is not present among all *Alicyclobacillus* spp. This finding has suggested that the fruit juice/beverage industry revise its quality control procedures to focus solely on guaiacol-producing *Alicyclobacillus* spp. Though peroxidase tests, GC and HPLC have been used to indicate the presence of guaiacol and/or guaiacol-producing *Alicyclobacillus*, there is no isolation media available for differentiating the ability to produce guaiacol. Thus, the purpose of this study was to develop a selective or differential media for isolating guaiacol-producing *Alicyclobacillus*. Forty-two different dyes were added at a concentration of 0.01% into K agar (pH 4.0). As a documented antimicrobial compound and the precursor of guaiacol, the potential of vanillic acid as a selective agent was also investigated. Two guaiacol producing (1101, 1016) and two non-guaiacol producing (C-GD 1-1, 19220) *Alicyclobacillus* isolates were streaked onto the agar media and color differentiation of the isolates were assessed. Guaiacol producing isolates formed purple irregular colonies with yellow background pigmentation while non-guaiacol producing isolates were inhibited after incubation at 43°C for 48 h. The new selective medium was named SK2 medium and consisted of 0.5 g peptone, 0.25 g yeast extract, 1.0 g glucose, 1.0 g Tween 80, 100 ppm vanillic acid, and 0.1% Chrome Azurol S per liter. The development of this selective medium provides the fruit juice industry with a simple alternative to monitoring guaiacol-producing *Alicyclobacillus*.

P4-04 DSC An Assessment of Airborne Contaminants in Beverage Processing Environments

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The past two decades have been characterized by a significant increase in the worldwide scientific database on airborne contaminants in various environments but little is known about the airborne contaminants composition of food-processing, food-service and beverage-processing establishments. The risk of contamination via the airborne route should, however, not be overlooked, but should be the beginning point in impediment of food contamination. New techniques and analytical methods have been used towards the precise identification of the sources of airborne dispersion, as well as in the evaluation of potential hazards. This paper focuses on indoor airborne contaminants as well as environmental factors influencing their distribution in South African beverage-processing environments. The microbiota assessed in the air included indicator organisms, fungi, and other Gram-negative and Gram-positive bacteria linked with food safety. The spread of airborne contaminants throughout the various sub-sections of the beverage environments is reported on, in addition to the influence of temperature, humidity, wind flow and airborne particulates. Relationships are explored between the airborne contaminants and certain indoor variables. Preventative measures, including proper monitoring of workers with any sickness, optimization of ventilation systems and drafting of airborne contaminants standards, particularly for bioaerosols, are recommended.

P4-05 DSC Ozonating Apple Cider to Reduce *Escherichia coli* O157:H7 and *Cryptosporidium parvum*

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Introduction: Outbreaks of *E. coli* O157:H7 and *Cryptosporidium parvum* have been linked to unpasteurized and ozonated apple cider. In order to preserve the integrity, eliminate pathogens, and increase the shelf life of unpasteurized cider, ozone treatment is being further investigated as an alternative to pasteurization.

Purpose: This study was initiated at the request of cider producers to determine the parameters necessary for ozonation of cider to obtain a 5 log reduction of pertinent pathogens.

Methods: Unpasteurized cider was obtained from Ohio cider producers. Apple cider (2.25 L) was filtered (99 um), inoculated with a cocktail of 5 strains of *E. coli* O157:H7 (10^8–10^9 CFU/ml) or *C. parvum* oocysts (106), and subjected to 180 min of ozone delivered at 200 ppm at 15 L per min (Marhoc Inc.). Samples (225 ml) were collected at 0, 60, 120, and 180 min and pathogens isolated by use of immunomagnetic beads (Matrix Microscience or Dynal). Beads were resuspended in sterile water and plated onto Sorbitol MacConkey (SMAC) and T-soy agar for bacterial analysis. Beads containing oocysts were assessed for viability using a human cell culture assay and PCR detection.

Results: A reduction of ≥ 5 log was determined for all samples treated for ≥ 60 min with ozone. There was no visible growth on T-soy or SMAC for these samples. Bacteria (2.0×10^6–3.0×10^7) incubated in non-ozonated cider survived with a 1–2 log reduction from initial inoculum. There was no notable change in oocysts in untreated samples. *Cryptosporidium* oocysts bound to beads, but were non-viable as determined by cell culture infectivity and PCR for sporozoite DNA.

Significance: The results of this study indicate ozone may be an effective method for the reduction of pathogens in apple cider; however, outbreaks have occurred in ozonated cider and it is important that processers are knowledgable about necessary ozone output concentrations.

P4-06 Survival of *Bacillus anthracis* Vegetative Cells in Beverages

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Little information exists about the behavior of *Bacillus anthracis* in foods. This study provides information about the survival of *B. anthracis* vegetative cells in beverages, including cola, clear sodas, apple and orange juices, bottled-water and milk. Beverages were inoculated with stationary phase *B. anthracis* (Sterne) (7~8 log CFU/ml) and stored at 4 or 20°C. In general, *B. anthracis* did not survive in soft drinks and juices and the rate of decline was faster when these beverages were stored at 20°C than at 4°C. *B. anthracis* declined most rapidly in cola. A 5–6 log reduction in population was observed in cola after 0.5 h at 20°C or 2 h at 4°C. There was a wide variation in results between replicates of other beverages; nonetheless, *B. anthracis* was inactivated by these beverages at a slower rate compared to cola. For example, the population of *B. anthracis* decreased by ca 4-log after 2 h in clear soda and juices at 20°C. In contrast, the cells were more stable in milk and water at 20°C compared to 4°C. After 7 days, the population of *B. anthracis* declined < 1 log (from 7.7 log to 7.1 log) in milk stored at 20°C. The population of *B. anthracis* dropped ca 3-log (from 7.7 log to 4.8 log) in milk after 3 days of storage at 4°C but only declined < 1 log (to 4.2 log) for the further 4 days of storage. In water, the population was reduced from 7.7 log to ca 6 log in the first 8 h and remained constant throughout the remainder of the 7-day storage at 20°C. Contrary to previous belief, our results indicated *B. anthracis* would survive in milk and water for a relatively long period of time. This could be potentially hazardous to human heath if subsequent sporulation occurred.

P4-07 Inhibitory Effects of Short Chain Fatty Acids against *Enterobacter sakazakii* in Laboratory Media and Liquid Foods

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Enterobacter sakazakii is an emerging foodborne pathogen that causes neonatal meningitis and sepsis, with mortality rates of 40 to 80%. Inhibitory effects of short chain fatty acids and their benefits have been known for years. This study was conducted to determine the effect of short chain fatty acids on inhibiting the survival and the growth of *E. sakazakii* in laboratory media and several liquid foods. Inhibitory effects and the minimum inhibitory concentration (MIC) of 9 acids (hydrochloric acid, lactic acid, malic acid, formic acid, phosphoric acid, propionic acid, citric acid, tartaric acid and acetic acid) against *E. sakazakii* were determined by the agar diffusion test and broth dilution methods. Out of 9 acids tested, 5 acids (malic acid, formic acid, propionic acid, citric acid, and acetic acid) showed antibacterial activity against one or more of *E. sakazakii* stains. In addition, propionic acid and acetic acid were found to be the most effective with the lowest MIC values of 16–31 and 31–63 mM, respectively, against *E. sakazakii*. Therefore, these two acids were selected for further testing in liquid foods (Sun-Sik, baby foods, grape juice and apple juice). Regarding foods tested, grape juice itself has an inhibitory effect and reduced or maintained levels of *E. sakazakii* during storage. However, 10 mM of propionic acid and acetic acid in Sun-Sik and apple juice, and 100 mM of propionic acid and acetic acid in baby foods, were necessary to eliminate *E. sakazakii* in each food type. These results suggest that short chain fatty acids such as propionic acid and acetic acid may possibly be used as preservatives to inhibit *E. sakazakii* in liquid foods.

P4-08 Inhibition of *Enterobacter sakazakii* by Some Naturally Occurring Organic Compounds in Combination with Nisin

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The use of natural antimicrobial substances has received increased interest because of concerns regarding the safety of synthetic compounds. *Enterobacter sakazakii* is an emerging foodborne pathogen that causes meningitis, bacteremia, sepsis, and necrotizing enterocolitis in neonates and children, resulting in a high mortality rate. The antimicrobial activity of natural organic compounds carvacrol, thymol, diacetyl, eugenol, and cinnamic acid alone and in combination with nisin on the growth of *E. sakazakii* in laboratory media was determined in this study. The minimum inhibitory concentration (MIC) of five natural organic compounds was determined and the effect of them in combination with nisin was evaluated by comparing treatment with each natural organic compound alone and in combination with 25 ppm nisin in tryptic soy broth. Among tested natural organic compounds, the MIC of carvacrol and thymol was 1.25 mM and showed the strongest inhibitory activity against *E. sakazakii*, whereas the MIC of cinnamic acid was higher than 5 mM and therefore showed the weakest inhibitory activity. The order of inhibition due to natural organic compounds was carvacrol = thymol > eugenol > diacetyl > cinnamic acid. However, the combination of each compound with nisin did not result in the enhancement of their antimicrobial activities except when nisin was combined with diacetyl. Therefore, the combined treatment of diacetyl and nisin showed a synergistic effect of preventing growth of *E. sakazakii*. This study shows the potential of natural organic compounds to control *E. sakazakii* in several types of foods as safe additives. However, studies in foods are required before they can be applied commercially.

P4-09 Inhibitory Effect of Green Tea and Rosemary Leaves against Foodborne Pathogens in Laboratory Media and Rice Cake

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In recent years, there has been an increasing interest in the use of natural tea or herbal plant materials in foods because of their functional

properties on health. In this study, the antimicrobial effects of green tea and rosemary against foodborne pathogens were determined in laboratory media and Asian-style rice cake. The growth of each pathogen (*Bacillus cereus*, *Salmonella* Typhimurium, *Enterobacter sakazakii*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes*) in tryptic soy broth (TSB) or rice cake with or without the addition of green tea or rosemary leaf flours before autoclaving or cooking, respectively, was investigated following inoculation. The addition of 1% green tea and rosemary showed similar results in inhibiting the growth of pathogens in TSB, although green tea was more effective at controlling *L. monocytogenes* than was rosemary. However, both plants showed inhibitory effects against all pathogens tested in this study. Green tea was particularly effective against *B. cereus*, *S. aureus*, and *L. monocytogenes*, and rosemary produced strong inhibition of *B. cereus* and *S. aureus*. The addition of 1 or 3% green tea or rosemary to rice cake did not significantly reduce total aerobic counts; however, levels of *B. cereus* and *S. aureus* were significantly reduced in rice cakes stored for 3 days at room temperature (22°C). The order of antimicrobial activities against *B. cereus* in rice cake was 1% rosemary < 1% green tea < 3% rosemary = 3% green tea. These results indicate that the use of natural plant materials such as green tea and rosemary could improve the microbial quality of foods.

P4-10 Comparative Efficacy of Alcohol-based Hand Sanitizers and Antibacterial Foam against Norovirus Using Fingerpad Method

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Introduction: Noroviruses are commonly associated with outbreaks of acute non-bacterial gastroenteritis, and hands are a principal vehicle of this transmission. Alcohol-based hand sanitizers and antibacterial foam handwashes are popular hand hygiene products, but little is known about their effectiveness against noroviruses on contaminated hands.

Purpose: We examined the efficacy of two alcohol-based hand sanitizers (a traditional, 62% ethanol commercial product and a new formulation based on 70% ethanol and a synergistic blend of polyquaternium-37 and citric acid), one commercially available antibacterial foam handwash (0.5% chloroxylenol active ingredient), and a hard water rinse control against Norwalk Virus (NV) on fingerpads of volunteers.

Methods: Using the ASTM (American Society of Testing and Materials) E 1838-02 standard method, samples were collected from 170 fingerpads of 24 subjects. Approximately 8.6 x 10^5 NV particles were inoculated on each fingerpad. NV RNA was extracted by a heat-release method and RNA titers were assayed by a one-step TaqMan Real-time quantitative RT-PCR.

Results: The 70% ethanol-based hand sanitizer, antibacterial foam handwash and water rinse resulted in an average of 1.46 (± 0.46), 1.53 (± 0.82) and 1.40 (± 0.34) log NV RNA reductions, respectively. All three hygiene methods provided a significant reduction of NV compared to baseline ($P < 0.001$), but were not significantly different from each other ($P > 0.05$). The 62% ethanol-based hand sanitizer reduced the NV titers by an average of 0.14 (± 0.31) log, and was not significantly different compared to baseline virus levels eluted from fingerpads.

Significance: These results demonstrate that handwashing with water and antibacterial foam are effective methods to remove NV from fingers. The results also show it is feasible for an alcohol-based hand sanitizer to produce significant NV kill on contaminated fingers. This new synergistically formulated hand sanitizer is therefore a viable option to reduce the spread and risk of noroviruses in food service or other settings.

P4-11 Inactivation of Viruses Using a Synergistically Formulated Alcohol-based Hand Sanitizer

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Introduction: The CDC estimates that norovirus is the leading cause of foodborne illness in the US, and reports indicate that hands are the most important vector for transmission. The 2005 Food Code does not recommend hand sanitizers for prevention of Norovirus transmission, likely because current products are relatively ineffective against norovirus surrogates.

Purpose: Evaluate novel hand sanitizer formulations for rapid virucidal activity against feline calicivirus (a surrogate for human norovirus) and other relevant enveloped and non-enveloped viruses.

Methods: Virucidal assays were performed by quantitative suspension test. Bacteriophage MS2 plaque titers were determined by soft agar overlay after infection of *Escherichia coli* ATCC-15597. Mammalian virus titers were determined by infecting appropriate host cell lines and monitoring cytopathic effects.

Results: Concentrations of up to 78% ethanol exhibited minimal activity against MS2. An extensive screening program showed that numerous other ingredients provided no significant increase in efficacy (≤ 1.0 log reduction, 1 min). The synergistic combination of ethanol, quaternary polymer and organic acid produced a 4.3 log reduction against MS2 in 1 min. A hand sanitizer formulated to contain 70% ethanol, polyquaternium and citric acid completely inactivated feline calicivirus in 30 s (> 4.75 log reduction); completely inactivated rotavirus, rhinovirus, influenza A, influenza B, Avian influenza (H5N1), and respiratory syncytial virus in 30 s; and reduced poliovirus > 3 log in 30 s. Some degree of efficacy was attained at shorter exposure times, and against mouse norovirus and hepatitis A virus.

Significance: Based on these results, we conclude that this new synergistically formulated ethanol hand sanitizer is a promising option for managing the transmission of viruses, including noroviruses, in food service settings. These types of control measures should be seriously considered as part of hand hygiene and overall restaurant infection control plans to reduce the transmission of foodborne viruses.

P4-12 DSC Comparative Susceptibilities of Hepatitis A, Feline Calicivirus, Coliphage MS-2, and Coliphage X-174 to Inactivation by Quaternary Ammonium and Potassium Peroxymonosulfate Disinfectants

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Introduction: Small, non-enveloped viruses such as picornaviruses and caliciviruses are extremely resistant to inactivation. Experiments to determine the susceptibility of these viruses to disinfectants is costly and time consuming due to the requirement for tissue culture to propagate and test them. Coliphages MS2 and X-174 are similar in size, construction, and nucleic acid composition to pathogenic viruses like hepatitis A and norovirus.

Purpose: The utility of bacteriophages MS2 and X-174 as surrogates for pathogenic viruses in disinfectant susceptibility testing was investigated. The susceptibility of hepatitis A, feline calicivirus (as a norovirus surrogate), MS2, and X-174 to a quaternary ammonium and potassium peroxymonosulfate disinfectant was determined.

Methods: Ten µl of viral or phage stock containing a 5% organic load of fetal bovine serum was deposited onto a sterile glass coverslip and dried. Two-hundred fifty µl of disinfectant was applied at various concentrations for 10 min. Disinfectant activity was halted by the addition of 750 µl of neutralizer. Surviving viral titer and phage levels were determined using titration in tissue culture and the phage plaque assay, respectively.

Results: Disinfection was considered effective if the virus or phage was reduced to below the detectable limit of the assay. The quaternary ammonium compound was ineffective against all of the viruses and the phage, even at ten times the suggested use concentration. The oxidative disinfectant was effective against all of the test viruses and phage at the recommended use concentration and was somewhat virucidal at one-half and one-quarter of the recommended use concentration.

Significance: Our results suggest that coliphages MS2 and X-174 are good indicators of the efficacy of quaternary and oxidative disinfectants on small, non-enveloped viruses such as picornavirus and calicivirus.

P4-13 Antimicrobial Efficacy of Peroxy Foam against Foodborne Pathogens, Yeasts and Molds

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The United States has a zero tolerance for both *Listeria monocytogenes* in Ready-to-Eat foods and *Escherichia coli* O157:H7 in meat. Yeasts and molds grow in food processing environments and cause spoilage in foods. The objective of this study was to evaluate the antimicrobial efficacy of peroxy foam against two foodborne pathogens, *Listeria monocytogenes* and *E. coli* O157:H7, and the spoilage yeast *Zygosacchromyces rouxii* and molds *Eurotium herbariorum*, *Eurotium repens* and *Eurotium rubrum*. Test cultures (7–8 log CFU/ml) were treated with peroxy foam preparation (229.2 water: 7.4 foam) for 30, 60, 90 and 120 s. Survival of *L. monocytogenes* and *E. coli* O157:H7 was determined using tryptone glucose yeast agar incubated at 35°C for 24 h. Survival of yeasts and molds was determined using potato dextrose sucrose agar incubated at 25°C for 5 days. Counts of *L. monocytogenes* were reduced by 6.9 log CFU/ml in 30 s. Counts of *E. coli* O157:H7 were reduced by 4.7 log CFU/ml in 120 s. Counts of *Z. rouxii* and *Eurotium* spp. were reduced by 6.2 log CFU/ml in 30 s and 4.3 log CFU/ml in 120 s, respectively. These results suggested that use of peroxy foam could be an effective tool of sanitation against the zero tolerance organisms *L. monocytogenes* and *E. coli* O157:H7, as well as spoilage yeasts and molds.

P4-14 Evaluation of the Antimicrobial Action of Physical and Chemical Sanitizer Agents in Commercial Sponges Used for Cleaning in Kitchens

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Introduction: The cleaning process of equipment and utensils in kitchens uses sponges to eliminate food residues. Part of these food residues stays adherent to the sponge surfaces, which together with the moisture retained in the sponges offer a favorable environment for microbial growth.

Purpose: To evaluate the efficiency of physical and chemical sanitizer agents in commercial sponges previously inoculated with populations (8 log CFU/ml) of *Staphylococcus* sp. and *Escherichia coli*.

Methods: The microorganisms were inoculated separately in Brain Heart Infusion (BHI) broth and later compared with tube number 5 of McFarland Scale. The commercial sponges were treated with this broth and submitted to the actions of hot water (1, 2, 3, 4 min); microwave oven (1100 w 1600 w, frequency 2450 MHz for 30, 60 and 120 s); peracetic acid (25, 50, 100, 150, 200, 300, 350 ppm for 1, 2, 5 min) and citric acid (100, 200 and 400 ppm for 2 and 5 min). Microbial counting was performed by the Petrifilm method.

Results: The results showed that hot water for 3 to 4 min and action of the microwave oven for 1 to 2 min, both at 85°C, completely destroyed the inoculated microorganisms. The peracetic acid was efficient for the total destruction of *E. coli* (50 ppm/1 min) and *S. aureus* (100 ppm/2 min). The citric acid was efficient

in the total destruction of *S. aureus* (200 ppm/2 min) but for *E. coli* required 400 ppm/5 min.

Significance: Hot water and treatment in a microwave oven were shown to be efficient methods of destruction. The efficiency of the acids was different for each type of microorganism, the peracetic acid being more efficient than citric acid. Also, acid concentration had more influence than contact time.

P4-15 DSC Degradation of Cellulose Produced by Shiga-toxin Producing *Escherichia coli*

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Cellulose plays a structural role in biofilm formation by conferring mechanical strength to the well-structured hydrophobic network. It also confers on bacterial cells, such as *Escherichia coli*, the ability to resist disinfection by sanitizers. This study evaluated several enzymatic and chemical agents for their abilities to degrade cellulose produced by Shiga-toxin producing *E. coli* (STEC) cells. Cellulose produced by the cells of six STEC strains were treated with cellulase (0.50, 2.12, and 3.75%), acids (2 and 4% acetic acid and lactic acid, respectively), and detergents (quorum yellow and Zep formula 7961) under appropriate conditions. Each experiment was set up with appropriate controls, duplications, and replications. Following the treatment, cellulose remaining on the surface of the STEC cells was quantified using a colorimetric assay. Results indicated that treatments with 3.75% cellulase reduced the overall mean cellulose produced by the cells of six STEC by 85%. Acetic and lactic acid at 2% concentration reduced the overall mean cellulose by 36% and 52%, respectively. Treatments with quorum yellow decreased the overall mean cellulose produced by the cells of six STEC by 80%, while the treatments with Zep formula 7961 reduced the overall mean cellulose by 92%. Longer treatments and higher concentrations significantly improved the efficiencies of the treatments ($P < 0.05$). All evaluated agents significantly reduced the amounts of cellulose produced by STEC cells ($P < 0.05$). However, some treatments were more effective than others. The study identified cleaning/sanitizing agents that can potentially control biofilm formation in the food processing environment.

P4-16 Efficacy of First, Third, and Fifth Generation Quaternary Ammonium Compounds against *Escherichia coli* O157:H7 and *Listeria innocua*

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Quaternary ammonium compounds (QAC) are common antimicrobial compounds used in hospitals and the food industry. Evolution of QAC had given rise to different generations of QAC with claims of superior efficacy, especially when used in an environment with hard water and soil residues. This study was designed to determine the efficacy of first, third, and fifth generations of QAC against *Escherichia coli* O157:H7 and *Listeria innocua* under different conditions. A general design of experiment was conducted with 4 factors with multiple levels (first, third, and fifth generation types of QAC; 100 and 400 ppm concentration of QAC; with and without a soil load; and 1, 5, and 10 min contact times). Stainless steel disks were inoculated with a cocktail of *E. coli* O157:H7 or *L. innocua* with or without a soil load, dried, and placed at the bottom of sterile centrifuge tubes prior to contact with the QAC for the selected times. Survivors were enumerated on appropriate agar media. The selected QAC were more effective in reducing *L. innocua* (2.3 log reduction/disk) compared to *E. coli* O157:H7 (0.9 log reduction/disk; $P < 0.05$). No differences were observed among the first, third, and fifth generation of QAC with regard to their efficacy against the microorganisms tested ($P > 0.05$). Higher concentrations of QAC and increased contact time increased the efficacy against the microorganisms tested ($P < 0.05$). However, addition of a soil load had no effect on the efficacy against the same microorganisms ($P > 0.05$).

P4-17 Inactivation of *Bacillus* Spores Using a Peroxyacetic Acid-based Sanitizer

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This study aimed, first, to develop a method to prevent germination of *Bacillus* spores during the process of application to representative food contact surfaces, and second, to evaluate the efficacy of an aqueous peroxyacetic acid based sanitizer at inactivating the spores. For the first objective, to ensure that the effects of the sanitizer were measured against only non-germinating spores, a method was developed whereby spores were suspended in 70% ethanol, loaded onto stainless steel and glazed tile coupons, and dried at refrigerator temperature overnight. For the second objective, after storage at ambient temperature for 5 or 10 days, dried spores were exposed to various concentrations (from 0.4% to 10%) of a peroxyacetic acid-based sanitizer. Spores were recovered on tryptic soy agar after 20 min heat shock at 80°C. For *Bacillus cereus* ATCC 21281 spores, after 5 days storage there was 0.63 + 0.13 log reduction on stainless steel coupons and 0.97 + 0.40 on glazed tile when 0.4% to 4% concentrations of the sanitizer were applied with 30 s contact time followed by neutralization. Likewise, there was a 3.42 vs 3.72 log reduction on the respective coupons with 5% sanitizer, and 4.42 vs 4.48 log reduction with 10% sanitizer. After 10 days storage, there was a 0.61 + 0.04 log reduction on stainless steel coupons compared with 1.12 + 0.02 on glazed tile with use of 0.4% to 4% sanitizer. There were 6.73 vs 4.54 log reductions with application of 5% sanitizer. With *Bacillus thuringiensis* ATCC 33680 spores at 0.4% concentration there was <1-log reduction. Using 5% concentration, there was a 4.46 log reduction after 10 days holding on stainless steel and a 1.96 log reduction on tile. When treated with 10% sanitizer, > 5-log reduction on stainless steel and 4.75 log reduction on tile were observed. These data suggest that the

peroxyacetic acid sanitizer, when used as described, has excellent sporicidal activity against the *Bacillus* spores studied.

P4-18 Mitigation of *Alicyclobacillus* spp. Spores on Food Contact Surfaces

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Introduction: The prevalence of *Alicyclobacillus* spp. and other spore-forming spoilage organisms in handling and processing environments presents a sanitation challenge to manufacturers of products such as juices and beverages. Normal sanitation regimes such as hot water sanitization are not always effective in inactivating the spores of these organisms, particulary from surfaces such as wood, although vegetative cells are readily destroyed.

Objectives: The objective of this study was to assess the efficacy of various chlorine dioxide sanitizing regimes on populations of *Alicyclobacillus* spp. on stainless steel, wood and plastic conveyor material.

Methods: Either high or low spore concentrations (10,000 CFU/mL or 100 CFU/mL) were inoculated onto stainless steel, plastic or wood coupons and challenged with sanitizer. After treatment, the coupons were placed in sterile sample bags, massaged with neutralizing buffer, and samples were plated on Ali agar (AA) for enumeration of surviving populations. Samples were also examined before and after treatment by scanning electron microscopy (SEM) to confirm destruction or removal of the spores by the treatment.

Results: Of the range of chlorine dioxide concentration/contact time regimes evaluated for all contact surfaces, the most effective concentration/time regime applied was 100 ppm/10 min. The logarithmic reduction of initial populations ranged from 0–4.5. The chlorine dioxide was least effective when applied to wood, resulting in the lowest observed reduction in spore numbers.

Significance: Previous studies have shown that chlorine is not effective against *Alicyclobacillus* spp. in food processing environments at reasonable usage levels. Chlorine dioxide is an alternative treatment to destroy microbial spores of *Alicyclobacillus* spp. in the processing environment, thus leading to a lesser likelihood of subsequent contamination by these spoilage organisms of the processed product.

P4-19 Field Assessment of Sanitation Management for Food Suppliers of School Foodservice in Korea

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Introduction: The standards of equipment and facility are not yet well established, especially for food suppliers of foodservice in Korea. To prevent foodborne disease outbreaks in school foodservice operations, an effective management system is necessary to improve the food safety performance of these suppliers.

Purpose: The purpose of this study was to propose an effective sanitation management system by assessing sanitation practices in food commodity treatment for food suppliers of school foodservice in Korea.

Methods: Field assessment was performed at a total of 23 vendors supplying agricultural products, meat products, seafoods, industrial products, rice cakes and crops located in Seoul and Gyeonggi Province. The assessment tools were developed based on the prerequisite program of HACCP system and Food Sanitation Act. A 93-item check list was used to evaluate the areas of facility, manufacturing/preparation facility and equipment, food materials storage, transport and recall, production, water, personnel, and inspection. The maximum score of each item was two and the score was expressed as a percentage of total possible scores from the applicable items for each business type.

Results: The average score of seafood vendors showed the highest score, 95.5%, because of HACCP requirements businesses. The scores of rice cakes and crops were 49.6 and 66.4%, respectively. The items scored relatively low were periodical evaluation of sanitizing effect in cleaning and sanitizing; sampling and sample-handling methods in receiving management; and periodical evaluation of cooperated business in raw material supplier management.

Significance: These results indicate the difficulties of assuring the safety of raw materials and lack of the systematic cleaning and sanitizing system. Therefore, for a self-managed sanitation system, guidelines should be provided to establish the sanitation management system, reflecting the characteristics of business types.

P4-20 Effects of Cetylpyridinium Chloride against *Escherichia coli* O157:H7 Biofilms on Stainless Steel

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Escherichia coli O157:H7 can form biofilms on food contact surfaces and is responsible for several outbreaks due to cross contamination in food processing plants. The objective of this study was to investigate the antimicrobial effects of cetylpyridinium chloride (CPC), a quaternary ammonium compound, against *E. coli* O157:H7 biofilms grown on stainless steel surfaces in different temperatures and culture conditions. A five-strain mixture of *E. coli* O157:H7 (10^8 CFU/ml) cell suspension was prepared and attached to stainless steel chips by immersing the chips in the cell suspension and incubating at 22°C for 4 h. Each chip was then transferred to tubes containing tryptic soy broth (TSB), 10% TSB, or minimal salts broth (MSB) and incubated at 12°C and 22°C for 2, 10 and 21 days. The CPC inactivation of biofilms was studied by immersing the chips in 0.01%, 0.05%, 0.5%, 1.0% CPC solutions for 2, 5, and 10 min. The inactivation of biofilms grown in all the three media were time and concentration dependent. There were significant reductions ($P \leq 0.05$) in total cell counts between treatment times at 2, 5, or 10 min and between each CPC concentration compared to the

control. The 21-day and 10-day biofilms were significantly ($P \leq 0.05$) more resistant to CPC than 2-day biofilms. Biofilms grown in MSB were more resistant to CPC than biofilms grown in TSB or 10% TSB. Concentrations of 0.05% and 0.1% CPC were sufficient to completely inactivate biofilms (2-day, 10-day, and 21-day biofilms) grown in TSB and 10% TSB respectively, whereas a concentration of 1.0% CPC was required to completely inactivate the 21-day biofilms grown in MSB. This study shows the potential use of CPC as a sanitizer to prevent *E. coli* O157:H7 cross contamination in food processing plants.

P4-21 DSC Application of Sanitizers, Disinfectants and Detergents to Reduce *Bacillus cereus* Biofilm on Stainless Steel Surfaces

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This study aimed to establish the effectiveness of washing and sanitation programs for reducing contamination of *B. cereus* in biofilms on stainless steel surfaces. Six sanitizers, QAC, alcohol, chlorine, calcium oxide, iodophor, and hydrogen peroxide, were evaluated. The detergent treatment which destroyed biofilm reduced 0.1 log CFU/chip of *B. cereus*. The water treatment did not destroy biofilm and the treatments of chlorine 200 ppm for 10 min and 300 ppm for 5 min, iodophor 200 ppm for 10 min, and QAC 400 ppm for 10 min eliminated all *B. cereus*. The *B. cereus* without biofilm was eliminated by chlorine 100 ppm for 10 min, 200 ppm for 5 min, and 300 ppm for 3 min; by iodophor 100 ppm for 10 min; and by QAC 400 ppm for 10 min. However, ethanol, hydrogen peroxide, and CaO did not eliminate all *B. cereus* regardless of biofilm formation. Therefore, detergent should be used prior to treatment of sanitizers and disinfectants for the purpose of controlling *B. cereus* on stainless steel surfaces.

P4-22 Novel Approach for Assessing the Efficacy of In-place Cleaning Methods for Food Equipment

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In Place Cleaning (IPC) of food equipment is vital for the delivery of safe food products in the retail food and restaurant sectors. IPC is defined as a method of cleaning and sanitizing equipment surfaces in their assembled form by mechanically circulating or passing a detergent solution, water rinse, and sanitizing solution (chlorine or quaternary ammonium) onto or over the surfaces. IPC efficacy standards have been developed and involve seeding the food equipment with a challenge organism, exposing the units to the sanitizer, and evaluating the reduction of the challenge organism in post IPC product. Current methods utilize chromogenic selective media for the enumeration of *E. coli*. Because of components of the selective media, this method may not effectively assess the injured but still viable population. The goal of this study was to design a method using non-selective media to aid in injured cell recovery, while still maintaining a unique appearance for the challenge organism versus any background bacteria. *E. coli* ATCC 11229 was transformed via electroporation with a plasmid vector (pUC19) containing a fluorescent protein (FP) and an ampicillin resistance gene. Two strains were generated: one expressed AcGFP1 (Aequorea coerulescens green FP) and the other expressed DsRed-Monomer (Anemonia sulcata red FP). A bench-top time kill study was performed to determine whether the engineered strains possessed the same sensitivity to chlorine and quaternary ammonium as the wild type. No significant difference was observed at any of the concentrations tested. The strains expressed the FPs on both selective and non-selective growth media indicating that a non-selective media may be an option for future IPC studies. The final phase of the study was to evaluate the performance of the FP strains against the wild type in an IPC application. At the time of this abstract these studies were in progress.

P4-23 Evaluation of Infrared Spectroscopic Methods for Analysis of Organic Soils on Stainless Steel Surfaces

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Cleaning-in-place (CIP) systems are extensively used in food and pharmaceutical industries with the purpose of complete removal of organic and inorganic soils, including biofilms. Validation of the achievement of these goals is critically important. Fourier-transform infrared spectroscopy (FT-IR) is recognized as the most sensitive optical absorption technique for detecting low concentrations of organic compounds on reflective surfaces and has great potential as a rapid cleaning validation tool. The objective of this study was to evaluate IR spectroscopic methods for analysis of organic soils on stainless steel surfaces with different finishes. Three IR spectroscopy techniques, attenuated total reflectance (ATR), a mid-IR grazing-angle fiber optics probe, and IR microspectroscopy with reflection and ATR accessories were evaluated for detection of organic matter on coupons (~2.5 x 2.5 cm) soiled with whole milk samples. An indirect method (ATR-FTIR) was evaluated using various swab types and sample concentration methods to obtain spectra with minimal interference resulting from the sample preparation. The mid-IR grazing angle fiber optics probe and IR micro-spectroscopy were the direct evaluation methods utilized. Indirect analysis methods yielded low absorbance levels and involved extensive sample preparation. Both grazing-angle probe and microspectroscopy allowed for differentiation of clean and soiled coupons. The surface finish and the direction of the grits affected the spectra obtained. Spectral definition obtained by IR microspectroscopy was superior to the mid-IR grazing-angle probe. On the other hand, the surface area used to collect spectral information with the microspectros-

copy was relatively small and several areas needed to be scanned to get a good representation of the sample distribution on the coupon. The mid-IR grazing-angle probe tested collected spectra from a larger surface area and allowed for in-situ examination of the samples. Infrared spectroscopy offers great potential as a rapid cleaning validation tool for monitoring the effectiveness of CIP systems to remove organic soils.

P4-24 Reduced Attachment and Biofilm Development of *Escherichia coli* on Nickel-silver Alloy and Copper Surfaces

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Introduction: Bacterial biofilm formation in food processing environments has important food safety implications. Bacteria attach to many different types of surfaces, but attachment surfaces may vary in their ability to support biofilm development due to different substratum characteristics.

Purpose: In this study, different surfaces (copper; nickel-silver alloy 1 containing ca. 73% copper, 21% zinc and 6% nickel; and nickel-silver alloy 2 containing 72% copper, 9% zinc and 19% nickel) were assessed for attachment by *E. coli* ATCC 8739 in comparison to stainless steel.

Methods: High concentrations (10^5) of bacteria were used to represent a "worst-case" scenario and inoculated onto metal coupons (4 cm^2) and incubated at room temperature (ca 20°C) for 7, 24 and 96 h. After incubation and rinsing, coupons were placed in Schott bottles containing glass beads and shaken to dislodge the cells. Aliquots were then plated onto tryptone soya agar using the droplet plate technique and colony forming units were counted. Further coupons were prepared for scanning electron microscopy by standard methods.

Results: The highest counts were always obtained on stainless steel surfaces at each time interval (5.5, 7.7 and 8.6 log CFU/cm^2, respectively). Fewer *E. coli* cells attached to both types of nickel-silver alloy coupons, by ca. 2–3 log CFU/cm^2, compared to stainless steel. However, counts were significantly lower ($P < 0.05$) on nickel-silver 1 only, which was attributed to higher concentrations of copper present in nickel-silver 1 than in nickel-silver 2. Significantly ($P < 0.05$) fewer *E. coli* cells attached to copper surfaces (1 log CFU/cm^2) than to stainless steel or the nickel-silver alloys. Scanning electron micrographs of nickel-silver alloy coupons showed shorter rods compared to those attached to stainless steel, indicating possible cell injury.

Significance: Results suggested that food contact surfaces containing nickel-silver alloys and copper may aid in reducing biofilm formation in food-processing environments.

P4-25 DSC Prevalence of *Escherichia coli* O157:H7 on and Potential Biotransfer of *Staphylococcus* and *Enterococcus* between Cleaning and Handling Tools and Ready-to-Eat Food Products in Retail Delicatessens

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Introduction: Previously we evaluated the microbiology of Ready-to-Eat (RTE) foods and associated handling and cleaning tools at 4 retail delicatessens in Johannesburg, South Africa and suggested that cleaning tools may represent a reservoir for contamination with potential foodborne pathogens.

Purpose: This study aimed to characterize selected Gram-negative and -positive populations isolated from handling and cleaning tools.

Methods: Two-hundred coliform and 300 *E. coli* colonies were randomly selected from countable Rapid' *E. coli* 2 Agar plates and further screened on BD BBL™ CHROMagar™ O157 for *E. coli* O157:H7. Twenty Gram-positive cocci were randomly selected from Baird-Parker Agar plus Egg Yolk Tellurite (0.5% w/v) plates and subjected to 16S rDNA sequence analysis by standard methods.

Results: No *E. coli* O157:H7 colonies were associated with RTE foods, cleaning tools, hands, or gloves. However, results of 16S rDNA sequence analysis indicated that several Gram-positive isolates were identified as *Staphylococcus aureus*, *S. pasteuri* and *S. sciuri*. *Enterococcus fecalis* strains were also found. The same strains were identified from cleaning and handling tools, and associated RTE foods. For example, strain 180 isolated from gloves worn by food handlers, and strains 166 and 111 associated with chicken mayonnaise filled baguettes and cut fruit salad, respectively, were genetically homologous (100%) to each other, and were identified as *S. aureus* (100% homology to *S. aureus* ATCC 14458). Similarly, strain 118 isolated from a cleaning cloth, and strains 112, 171 and 172 associated with various salads, were genetically homologous (100%) to each other, and were identified as *E. fecalis* (100% homology to *E. fecalis* ATCC 19433). Thus, it appeared that transfer of Gram-positive populations may have occurred between cleaning tools, hands, gloves, and RTE foods.

Significance: Transfer of bacteria, in particular potential pathogens, such as *S. aureus*, to RTE foods from cleaning and handling tools may hold food safety implications.

P4-26 Comparison of Sanitary Designs of Delicatessen Meat Slicers

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The cleanability and sanitization of meat slicers used in delicatessens are very Important, as designs that entrap foods that could harbor food residues and may allow foodborne pathogens to thive. The objective of this project was to compare the sanitary

designs of different meat slicers commonly used in delicatessens. Four models from three brand name meat slicer manufacturers were selected for study based on a market survey. Oven-roasted turkey breast was sprayed with Clue Spray,™ which releases green fluorescence under UV light. A half chub of Clue Spray™-sprayed turkey breast was sliced on each slicer. The sanitary conditions of all surfaces and parts were determined by visualizing the slicers under regular room light and UV light (365 nm wavelength) after cleaning without disassembling each slicer and cleaning the disassembled units. There were substantial differences in the sanitary characteristics of the four slicers. Carriage trays having a flat, simple bottom design were considerably easier to clean than those with compartments or ridges. The distance between the lower edge of the blade guard ring and the base of a slicer should be large enough to allow contact by hands and brushes. Simple and smooth blade covers were the best for sanitary design compared to those with bolts, ridges and rough surfaces, and complex in appearance. Blades with fewer indentations also were easier to clean. Blade guard rings having a sharp and deep inner side were difficult to clean. High ridges near surfaces at the slicer front and back made cleaning difficult. Meat grips with fewer teeth per square centimeter were best for cleanability. Surfaces with large grooves and smooth small ridges were better designs compared with surfaces with small grooves and large ridges. Grooves with gradual endings were more easily cleaned than grooves with abrupt endings. All slicers evaluated had some desirable features. Combining these desirable features into a redesigned slicer could provide a greatly improved meat slicer from a sanitation perspective.

P4-27 DSC The Role of Biofilm Formation in the Protection of *Enterobacter sakazakii* from Chlorination

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Chlorination is known as an effective method for surface and equipment disinfection in production plants, hospitals and households. Chlorine concentrations of disinfectant solution varies from 50 ppm to 200 ppm. Different studies report that the attachment of microorganisms to surfaces followed by biofilm formation enhance the resistance of cells to sanitizers. However, little is known about *E. sakazakii* biofilm resistance to chlorine. We were interested in studying the effect of chlorination over *E. sakazakii* cells that attach and produce biofilms on different surfaces commonly used for the preparation and administration of infant formula. Two reference strains, *E. sakazakii* ATCC 29004 and 29544 from the American Type Culture Collection (ATCC) were used to inoculate coupons of each of the following materials: stainless steel, silicone, polypropylene and glass. After three hours of incubation at 35°C the inoculated coupons were rinsed with phosphate buffer pH 7.0, transferred to sterile petri dishes containing sterile nutritional formula (Portagen, Mead Johnson), and incubated for 48 h at 35°C. After biofilm growth, the coupons were washed to remove unattached cells and placed in a petri dish filled with 20 ml of phosphate buffer, 20 ml of 100 ppm chlorine solution or 20 ml of 200 ppm chlorine solution for 30 min. Survivors were reported as log CFU/cm ≤. The experiment was replicated three times. The results obtained indicate that once *E. sakazakii* attaches to the surfaces of study, it produces biofilms, making it resistant to the bactericide effect of solutions with chlorine concentration as high as 200 ppm free chlorine; the subsequent sloughing off from the production surfaces would explain the contamination of the formula and appearance of outbreaks.

P4-28 Production of Bacterial Biofilms by *Enterobacter sakazakii* and Other *Enterobacteriaceae* on Neonatal Enteral Feeding Tubes

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While the microbial safety of powdered infant formula has received much attention, due to *Enterobacter sakazakii*, the possibility of bacterial biofilms on the neonatal enteral feeding tube acting as a loci for infection has not been thoroughly investigated. Clumps of bacterial cells can be dislodged from biofilms, and may survive the acidic neonatal stomach. This study addressed this issue using a total of 23 strains of organisms identified by FAO-WHO (2004 and 2006) as neonatal infectious organisms, that may also be isolated from powdered infant formula. These were: *Enterobacter sakazakii*, *E. cloacae*, *Citrobacter freundii*, *C. koseri*, *Pantoea* spp., *E. coli*, *E. hermanii*, *E. vulneris*, *Klebsiella oxytoca*, *K. pneumoniae*, *Hafnia alvei*, and *Acinetobacter baumanii*. The majority of these strains had previously been isolated from powdered infant formula. Five types of enteral feeding tubes were investigated. These varied in composition, including antimicrobial silver-impregnation, and diameter. A number of organisms, including *E. sakazakii*, are noted for their production of extracellular, capsular polysaccharides which could act as an adherence factor. Therefore, capsulation was assessed according to colony morphology on high sugar agar plates (XLD and milk agar). Organisms attached to the tubing as biofilms were enumerated in situ by use of the direct impedance method. The cell density was determined after incubation with the tubing for 24 h at 37°C. All organisms formed biofilms which could not be removed by vigorous washing. The biofilm cell densities varied. For example, on PVC tubing the biofilms were 10^3, 10^5 and 10^7 CFU/cm, for *H. alvei*, *E. sakazakii* ATCC 12868 and *E. vulneris* respectively. *E. sakazakii* NCTC 11467T (non-capsulated) formed less dense biofilms than the other strains of *E. sakazakii*. The biofilm on the "antibacterial" tubing was not significantly lower than that of ordinary enteral feeding tubes.

P4-29 Comparison of Effectiveness of Various Sanitizing Agents by Different Validity Test Methods

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This research was conducted to compare effectiveness of various sanitizing agents by application of different test methods such as "Suspension Test Method" which is the official method in Korea and "Quantitative Surface Test Methods" which are officially used in United States and European Unions. Each of 2 different commercial products from 5 kinds of sanitizing agents, chlorine, iodine, quaternary ammonium, alcohol, and peroxide compounds, was evaluated for effectiveness against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeurginosa*, and *Enterobacter hirae*. The evaluation methods were followed as Korean Food Additive Code, CEN EN 13697, ASTM E 2197-02, and ASTM E 2111-00, respectively. As "Suspension Test Method" and ASTM E 2111-00 method were used, all 10 commercial products caused a decrease of more than 5 log and 4.1~6.1 log of microorganisms, respectively, which result in "Pass" to their standards and specifications. However, in the case of using the CEN EN 13697 method, only 4 commercial products decreased by 5.2~7.0 log and others could not pass the standards and specifications. Sanitizing agents containing chlorine and quaternary ammonium compounds showed poor effectiveness, which were 1.7~2.9 log reductions. *Staphylococcus aureus* showed strong resistance to those sanitizing agents. Six commercial sanitizing agents decreased by 4.5~6.0 log, which are "Pass" to the standards and specifications when ASTM E 2197-02 was applied. The sanitizing agents containing iodine compound showed the least effectiveness, which were 1.8~2.8 log reductions. In conclusion, "Quantitative Surface Test Methods" may be tighter than "Suspension Test Method" in terms of conformance test on effectiveness of sanitizing agents.

P4-30 A Comparative Analysis of Proficiency Testing Results from Food Laboratories

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Introduction: In order for meat processors to demonstrate compliance with regulations such as the "zero-tolerance" requirement for *Escherichia coli* O157:H7 in raw ground beef and performance standards for *Salmonella*, pathogen screening programs are widely implemented using commercially available rapid test kits. The USDA-FSIS Microbiological Laboratory Guidebook (MLG) recommends that these kits meet the minimum performance criteria with sensitivity and specificity rates of: (i) ≥98% and ≥90% for *E. coli* O157:H7 and, (ii) ≥97% and ≥90% for *Salmonella*. Laboratories utilizing these rapid test kits often participate in proficiency testing programs as part of a quality control system.

Purpose: This comparative study was conducted to review the results available for proficiency testing in food testing laboratories and to evaluate compliance with recommended performance criteria.

Methods: Sensitivity and specificity rates were calculated from published proficiency testing results for the seven most widely used rapid test kits for the detection of *E. coli* O157:H7 and *Salmonella*. Confirmation methods were not considered in the analyses.

Results: The most commonly used test kits for the detection of *E. coli* O157:H7 demonstrated a range of sensitivity rates from 77.4 to 100% and a range of specificity rates from 90.5 to 100%. *Salmonella* detection kits showed a range of sensitivity rates from 76.5 to 100% and a range of specificity rates from 95.3 to 100%. Sensitivity rates of the majority of methods considered were lower than the minimum performance criteria recommended in the USDA-FSIS MLG.

Significance: These results suggest that the rapid test kits considered, as implemented by independent laboratories, do not meet the performance criteria recommended by the USDA-FSIS.

P4-31 Efficacy of Sanitizing Program to Control *Listeria* in Poultry Facilities

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Controlling the presence of *Listeria monocytogenes* (Lm) in the food processing environment is one of the most difficult tasks that companies have to face. It requires, among other things, the use of efficient chemical agents together with training the workers regarding the importance of these operations to improve their performance. Some companies have opted for monitoring *Listeria* sp. instead of Lm. The aim of this study was to evaluate the efficiency of a cleaning-sanitizing program in diminishing *Listeria* presence in a poultry facility. The first step was to evaluate the pre-existing GHP (good hygiene practices), using a check list. Environmental and air samples were collected 3 times and analyzed for *Listeria* presence according to ISO method. A new cleaning-sanitizing program was prepared and used for 45 days and a new evaluation was conducted. Samples from the same spots were collected (3 times) and reexamined. Lm isolates were submitted to PFGE. The results showed that after implementing the new procedure *Listeria* overall contamination decreased from 48% to 11%. The lowest decrease rate was observed in the air samples, since the cooling system had design problems and could not be disassembled for cleaning. We believe that drastic reduction of *Listeria* contamination in this plant can be obtained if the company adheres to the use of high performance sanitizing products and follows the recommended use. The total involvement of the sanitizing team and managers is also required.

P4-32 Microbial Risk Factors Associated with Condensation in Ready-to-Eat Processing Facilities

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Processing plant environments are conducive to the accumulation of condensation on various surfaces, which can drip directly into food or onto food contact surfaces. Recently, the USDA has asked processors to address condensation in the HACCP plan; however, there is a lack of data describing the actual microbial risk associated with it. The objectives of this study were to determine the microbial risk associated with condensation in Ready-to-Eat (RTE) processing environments and to identify controllable risk factors associated with condensation formation. A minimum of 30 samples pre-operational and 30 samples during operation were collected from areas of visible condensation, overhead pipes and dripping pans in 3 RTE meat processing plants each season during a one-year period. Samples were weighed to quantify actual amounts of condensation present, after which samples were subjected to enumeration of Total Plate Counts, enterococci, *E. coli*, yeast and molds and detection of *Listeria* spp. Total aerobic plate counts ranged from non-detectable to 2.5 log CFU/ml, with most samples containing fewer than 100 cells/ml. The vast majority of samples did not contain any detectable *E. coli* or enterococci. Yeast and mold counts were less than 1.0 log CFU/ml in all samples. *Listeria* spp. was detected in only 3 samples of more than 600 that were analyzed. All were detected in operational samples. Data from this project will help processors to make science-based decisions on the risks associated with condensation in RTE plants and to establish CCP or monitoring methods needed in the HACCP plan.

P4-33 DSC A Vertically Integrated Colorimetric Rapid Detection Test for *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a ubiquitous pathogen and a major cause of food related illness. Current methods of detection are slow, laborious, and require expensive equipment, or technical expertise.

Purpose: A rapid, easy to use test to detect *L. monocytogenes* was developed, that requires little to no equipment or technical training.

Methods: The Phast Swab is a self-contained test device, containing a sampling tool (swab), growth media, immunomagnetic separation (IMS) beads, and a colorimetric substrate which, when cleaved by the enzyme phosphatidylinositol-specific phospholipase C (PI-PLC), forms a visible indigo reaction. A five-strain cocktail of *L. monocytogenes* was prepared so that the final concentration of the cocktail was 10^9 CFU/ml. The cocktail was serially diluted (10-fold) in a dilution range from 100–10^{-9}, and individual slices of deli turkey meat (100 mm diameter) were inoculated with 1 ml of each dilution. The meat was allowed to dry, and then each piece was swabbed with an individual Phast Swab, followed by a 10-h enrichment. Any *L. monocytogenes* cells present in the growth media were isolated and concentrated by IMS and the growth media was removed. Bacterial lysis buffer was added followed by the enzyme substrate, which reacted with PI-PLC to form the indicative indigo color for positive identification.

Results: The Phast Swab was capable of detecting 102 cells on the deli meat within 15 h. The test detected 55 of 57 *L. monocytogenes* tested and none of the non- *L. monocytogenes* tested. In pure culture, as few as 10^7 CFU/ml were directly detected within 3 h. The addition of 428 mM KCl to the growth media significantly increased the sensitivity of the assay.

Significance: These data show that the Phast Swab can detect *L. monocytogenes* on deli meat in a rapid and specific manner without the need for instrumentation to read the test result.

P4-34 Sterility Testing of a Dispensing Valve for Aseptic Function in Foodservice Applications

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Introduction: Manual dispensing apparatus for aseptically-packaged beverages or foods requires refrigeration of the package following breakage of the hermetic seal. The foodservice industry would benefit greatly by implementing a dispensing apparatus that maintains the sterility of products after continued use without the need for refrigeration.

Purpose: The purpose of this study was to evaluate the ability of a valve, designed to operate aseptically and dispense product with or without refrigeration, to maintain sterility of product following breakage of the hermetic seal and continued use simulating that of foodservice.

Methods: Plastic packages equipped with the "aseptic" dispensing valve in a bag-in-box (BIB) format were aseptically filled with enrichment media with and without the addition of 1% starch to simulate high and low viscosity products, respectively. BIB filled with media were left uninoculated or inoculated (10^4 CFU/ml) with *Staphylococcus aureus* or *Aspergillus niger* on the interior of the spout 1 cm from the opening to simulate consumer misuse. Uninoculated and inoculated BIB were stored at 25°C and media was dispensed twice a day, every five days, for up to 30 days to simulate foodservice use. Dispensates were observed for turbidity (compared to controls) indicating growth in BIB and, thus, breach of sterility. Growth was confirmed by standard plating techniques.

Results: There was no breach in sterility until day 25. At day 25, uninoculated BIB (1/45 samples positive for growth) containing high-viscosity media and BIB inoculated with *S. aureus* (1/45 samples positive for

growth) containing low-viscosity media became turbid. Viscosity and type of organism did not appear to influence the ability of the valve to maintain sterility for ≥ 20 d.

Significance: The results of this study provide evidence that the dispensing valve tested can maintain sterility of aseptically filled products following breakage of the hermetic seal and continued use under unrefrigerated conditions.

P4-35 Withdrawn

P4-36 DSC Sensitizing Processing-resistant Foodborne Spoilage and Pathogenic Bacteria to Ultra-high Pressure by Food Colorants

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Treating foods with ultra-high pressure (UHP) causes significant inactivation of their microbial load; however, a small number of survivors are often observed after extreme pressure treatments (a tailing phenomenon). Consequently, it is important to identify conditions and additives that enhance the efficacy of pressure against foodborne pathogenic and spoilage microorganisms. Based on preliminary work, it was hypothesized that food colorants could be used in combination with UHP to enhance pressure lethality. The seven US certified food colorants (FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6) were tested for synergy with UHP against *Lactobacillus plantarum* in citrate-phosphate buffer (pH 7.0). FD&C Red No. 3 was the only food colorant that exhibited antimicrobial properties as well as enhanced UHP lethality. Further studies were performed using FD&C Red No. 3 against processing-resistant *Listeria monocytogenes* and *Escherichia coli* O157:H7 strains. Synergy of inactivation occurred with FD&C Red No. 3 concentrations as low as 3 ppm when the pathogens were treated with 400 MPa for 3 min. FD&C Red No. 3 (10 ppm) and UHP (400 MPa for 3 min) inactivated a large population (> 7 log CFU/ml) of the processing-resistant *L. monocytogenes* OSY-328; UHP treatment alone inactivated ~0.3 log CFU/ml, whereas dye treatment alone inactivated 1.8 log CFU/ml. Gram-positive strains were sensitive to FD&C Red No. 3 alone, while *E. coli* O157:H7 was affected only when treated with the colorant in combination with UHP. These data suggest the role of the outer membrane of Gram-negative organisms as a protective layer that can be disrupted with UHP, resulting in sensitization of the bacterium to chemical treatments.

P4-37 Mechanism of Action of Antilisterial Peptides Produced by *Lactobacillus sakei* 2a, a Bacteriocinogenic Strain Isolated from a Brazilian Meat Product

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Introduction: Bacteriocins produced by lactic acid bacteria (LAB) are gaining increased importance due to their activity against undesirable microorganisms, especially *Listeria monocytogenes*, an important psychrotrophic foodborne pathogen. In previous reports, we have shown that strain *Lactobacillus sakei* subsp. *sakei* 2a, isolated from a Brazilian pork meat product, presents in vitro and in situ antilisterial activity. We also demonstrated that *L. sakei* 2a produces sakacin 2a and at least other eight anti-listerial peptides, all active against *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Lactococcus lactis*, *Enterococcus hirae*, *Enterococcus canis*, *Listeria monocytogenes* and *Listeria inoccua*. It is known that Class II bacteriocins act by altering the permeability barrier of the cellular membrane of the target cells, and one of the common mechanisms of inhibition is the dissipation of the membrane potential.

Purpose: The purpose of this study was to evaluate the mechanism of action of the antilisterial peptides produced by *L. sakei* 2a.

Materials and Methods: After purification, the peptides were tested for dissipation of membrane potential and depletion of intracellular pH of *L. monocytogenes*, using fluorescent probes, 3,3 dipropylthiocarbocyanine iodide [DiSC3(5)] and 2,7-bis-(2carboxyethyl) 5 (and 6)carboxyfluorescein (BCECF), respectively (Molecular Probes). The increase of fluorescence of treated *L. monocytogenes* cells was measured using a Cary Eclypse Varian spectrofluorimeter.

Results: All peptides decreased the membrane potential and dissipated the ΔpH of *L. monocytogenes* in a concentration-dependent fashion: 10 nM of sakacin 2a caused the same effect as 100 nM of any of the other peptides.

Significance: These results indicate that the antagonist activity of *L. sakei* subsp. *sakei* 2a can be attributed to different peptides presenting similar mechanisms of action, the same as described for Class II bacteriocins.

P4-38 Isolation and Purification of a Novel Bacteriocin from *Enterococcus* spp. with Broad Spectrum Inhibitory Activity

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Campylobacter jejuni is a Gram-negative human foodborne pathogen of primary importance. Poultry are frequently contaminated with *C. jejuni* during production, with the majority of commercial US flocks positive for the organism by the time the birds reach market age at about 6 weeks. There are currently few effective on-farm interventions for reducing colonization of *C. jejuni* in the poultry gut. We report the isolation and purification of a 5,362 Da bacteriocin produced by an *Enterococcus* species isolated from chicken ceca with broad spectrum activity against both Gram-positive and Gram-negative bacteria. The

bacteriocin, E-760, was isolated and purified by cation exchange and hydrophobic interaction chromatography. In vitro assays demonstrated that E-760 inhibits the growth of *Salmonella* Enteritidis, *S. choleraesuis*, S. Typhimurium, *S. gallinarum*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii*, *Staphylococcus aureus*, *S. epidermidis*, *Listeria monocytogenes*, *Campylobacter jejuni* and 20 other *Campylobacter* spp. isolates. Administration of bacteriocin E-760 treated feed significantly ($P < 0.05$) reduced colonization of young broiler chicks experimentally challenged with two strains of *C. jejuni* by more than 8 log CFU. Bacteriocin E-760 also significantly ($P < 0.05$) reduced colonization in the poultry gut of naturally acquired *Campylobacter* spp. in market age broilers when administered in treated feed for four days prior to analysis. Bacteriocin E-760 could be developed as a practical on-farm intervention for reducing consumer exposure to pathogenic *Campylobacter*.

P4-39 DSC Bacteriocin Structural Genes and Virulence Determinants among Bacteriocin-producing Enterococci Isolated from Different Food Samples

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Fifty-one bacterial isolates that produce antimicrobial agents were obtained from different food samples. Fourteen of these isolates produced antimicrobial agents that were active against *Listeria monocytogenes* and *Bacillus cereus*; these 14 isolates were selected for further investigation. Based on biochemical testing and 16S ribosomal RNA gene sequence analysis, these isolates were all identified as *Enterococcus faecalis*. The properties of the corresponding antimicrobial agents were characterized. Furthermore, the presence of genes encoding known bacteriocins and virulence factors were investigated by polymerase chain reaction (PCR). The structural gene encoding enterocin AS-48 was detected in twelve of the fourteen isolates, but not from OSY-5B1 and OSY-RM1. Nucleotide sequence of one of the twelve isolates showed 97.5% similarity to the gene encoding for enterocin AS-48, and the predicted amino acid sequence was 96.7% similar to the mature enterocin AS-48 protein. The fourteen isolates were also found encoding some or all the frequent combination of *Enterococcus* virulence factors. However, the *esp* gene, encoding an extracellular surface protein, was not detected in any of the fourteen isolates. The raw cow's milk isolate, OSY-RM1, was further studied. The antimicrobial agent produced by OSY-RM1 resemble class-II bacteriocins, and it was designated as Enterocin OSY-RM1 (Ent OSY-RM1). The plasmid-free *E. faecalis* OSY-RM1 mutant retained its antimicrobial activity, suggesting that Ent OSY-RM1 was encoded by chromosome-borne genes. The molecular mass of partially purified Ent OSY-RM1, based on SDS-PAGE analysis, was 4.0-4.5 kDa. In spite of the undesirable properties commonly associated with this lactic acid bacterium, the bacteriocin-producing *E. faecalis* OSY-RM1 is potentially useful in food applications.

P4-40 DSC Extraction of Nisin from a 2.5% Commercial Nisin Product Using Methanol and Ethanol

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Nisin is a class Ia bacteriocin used in the food industry to inhibit the growth of Gram-positive spoilage and pathogenic microorganisms in various food products. The antimicrobial effectiveness of the peptide can vary significantly depending upon the food application. One method that has been investigated for improving the activity of nisin is encapsulation. For successful encapsulation, purified nisin is preferred, though commercial nisin preparations contain only 2.5% nisin. The exclusion of dairy proteins and salts using purification procedures would thus constitute a potential advancement in the utility of the antimicrobial peptide. The primary objective of this study was to use solutions of methanol and ethanol to extract and purify nisin from a commercial powdered 2.5% nisin preparation. Commercial powdered nisin was mixed with 10–100% absolute ethanol or methanol at a concentration of 1000 IU/ml and gently agitated for 0.5–8 h. Following extraction, samples were centrifuged and supernatants were tested for nisin activity and protein concentration. A standard bioassay was employed to estimate the antimicrobial activity of nisin extracts against the indicator *Micrococcus luteus*. Approximately 75% nisin activity was recovered with 10% or 50% ethanol, compared to less than 1% at an ethanol concentration higher than 90%. Extraction with 10% or 50% methanol was similarly effective to comparable ethanol treatments. However, yields were significantly greater for extraction with methanol at concentrations greater than 90%. Fold purification for an 8 h extraction using 10% or 50% ethanol was 1.36 or 1.93 times, respectively. Purification was less than 0.1-fold at higher ethanol concentrations due to poor extraction. For methanol treatments, fold purification factors were 1.09 to 5.98 and increased as methanol concentration increased. This research demonstrates a method to extract and concentrate nisin that may provide an alternative means for applications such as encapsulation.

P4-41 In Vitro Assessment of Novel Edible Coats with Antimicrobial Features: Applications to Cheese

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A new generation of food packages is on the way, as traditional concepts have been hampered in their ability to further extend shelf life of food products, coupled to considerably high ecological footprints. One element of such a generation takes advantage of coats that inhibit undesirable microbial growth; however, most commercial solutions already available in the market include only antifungal compounds, which cannot be ingested. Edible coatings based on milk proteins, viz. whey proteins, have meanwhile been developed for use as protective layers in a variety of foods. Unfortunately, their main functionality relates

to barrier properties, so there is large room for improvement in appearance. Development of edible coats exhibiting general antimicrobial properties, and specifically designed for cheese applications, is thus an issue of practical relevance; however, a general solution may not exist, as different spoilage/pathogenic microflora have been associated with the cheese surface. Consequently, the objective of this research effort was to assess, via in vitro tests, the efficacy of several formulated antimicrobial edible coats, based on whey protein isolates as base coating material, and glycerol as plasticizer. The antimicrobial activity of several antimicrobial agents, such as a polysaccharide (chitosan), organic acids (lactic and citric acids), a bacteriocin (nisin) and lactoperoxidase, were tested against yeasts (e.g., *Yarrowia lipolytica*) and bacteria (e.g., *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*), previously isolated from cheese. The inhibitory effect of said antimicrobials was initially tested in culture media and in edible coats, which remained in contact with said microbial strains for 24 h. Afterwards, the best antimicrobial systems were spread on the surface of sliced hard and soft cheeses deliberately contaminated with target microorganisms (at an inoculum level of 10^5 CFU/ml), and the associated inhibitory activity was monitored. Lactic acid (at 6 g/L) yielded the strongest effect under the conditions tested against *Salmonella* spp. and *E. coli*. Different coatings showed different antimicrobial behaviors, depending on the microbial agent in stake; however, association of antimicrobial agents increased (as expected) the individual antimicrobial activities.

P4-42 Role of Crude Canola Extracts on *Listeria monocytogenes* Cell Invasion to CaCo-2 Cell Line

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Adhesion and invasion of *Listeria monocytogenes* to intestinal cells is an important initial step in the pathogenesis of infection, and research suggests that some proteins, including those involved in internalin and actin polymerization, are responsible for the adhesion and invasion. This study investigated the role of several cultivars of crude canola extracts on the growth, cell adhesion and invasion of *L. monocytogenes* to human intestinal enterocyte-like Caco-2 cell lines. Tryptic soy broth (TSB) supplemented with four varieties of crude canola extracts at different concentrations (0.25, 0.5, 1, 2, 50 and 100%) were inoculated with *L. monocytogenes* Scott A and incubated overnight at 35°C. The growth rates were recorded every 2 h for a total of 24 h to determine the inhibitory effect of canola extracts. The inoculate extracts were tested for ability to adhere to and invade Caco-2 monolayers in 24-well tissue culture plates, using a cell invasion and cell adherence assay. Our results indicated that higher concentrations (50 and 100%) of canola extracts significantly ($P \leq 0.05$) affected the ability of *L. monocytogenes* to grow and invade Caco-2 cells but had no effect on the ability to adhere (73°C 83%) to Caco-2 mammalian tissue culture cells, which was consistent with the control (75%). *L. monocytogenes* grown in 100% canola had significantly ($P \leq 0.05$) longer lag phases (5 – 8 h) compared to the control (3 h). The ability of *L. monocytogenes* Scott A to invade intestinal cells was lower (25 to 30%) compared to the control (43%), suggesting that the growth of the culture may have affected cell invasion, since there was a significant reduction in growth rate in the presence of crude canola extracts. These results strongly indicate that different varieties of canola extract may play an important role in modulating cell invasion and the cell invasion phenotype, and the use of canola extracts may be an alternative for reducing the virulence of *L. monocytogenes*.

P4-43 Moved to P3–08

P4-44 Antimicrobial Activity of Citrus-based Natural Extracts against *Escherichia coli* O157:H7

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Seven citrus-based natural antimicrobials were evaluated for their ability to inhibit the growth of *E. coli* O157:H7 (ATCC 43889, 43890, and 43895). Zones of *E. coli* O157:H7 inhibition by the citrus-derived fraction (10 µl /6 mm disk) was determined by a disk-diffusion assay on sorbitol-MacConkey agar. Inhibition zones were observed after 48 h long growth of *E. coli* O157:H7 cells at 37°C. Orange CP VAL terpeneless FAB 968611 and Limonene 1 x Dist FAB 955430 inhibited *E. coli* O157:H7 with inhibition zones of approx. 11–24 mm dia. The remaining other five citrus-derived extracts (Orange Oil FL VAL 1121 ARR 974760, Orange 5 x Conc VAL 4121 ARR 968374, Orange terpenes ESS 1120 ARR 986259, Orange terpenes CP 1100 ARR 986255, and Orange terpenes OEO HP 1100 ARR 986257) were weakly inhibitory to *E. coli* O157:H7, yielding inhibition zones of 7–11 mm. dia. These studies show citrus-derived natural compounds differ in their inhibitory activity against *E. coli* O157:H7, and some have potential applications as inhibitory agents against *E. coli* O157:H7.

P4-45 Ciprofloxacin-sensitive and Ciprofloxacin-resistant *Campylobacter jejuni* Inhibition by Citrus-based Natural Extracts

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Six citrus-based natural antimicrobials were evaluated for their ability to inhibit the growth of ciprofloxacin-sensitive (UAF 244) and cipro-floxacin-resistant (UAF 199) *Campylobacter jejuni* obtained from retail raw chicken carcass rinses. Zones of *Campylobacter* inhibition by the citrus-derived fraction

(10 µl /6 mm disk) were determined by a disk-diffusion assay on *Campylobacter* agar. Inhibition zones were observed after 48 h long growth of live spiral *Campylobacter* cells at 42°C under microaerophilic conditions. Orange CPVAL terpeneless FAB 968611 was found to be highly inhibitory to both ciprofloxacin-sensitive and ciprofloxacin-resistant *C. jejuni*, yielding maximum inhibition zones of 48–56 mm dia. Limonene 1 x Dist FAB 955430 yielded *Campylobacter* inhibition zones of 13–16 mm dia. against both *C. jejuni*. The remaining other four citrus-derived extracts (Orange Oil FLVAL 1121 ARR 974760, Orange terpenes ESS 1120 ARR 986259, Orange terpenes CP 1100 ARR 986255, and Orange terpenes OEO HP 1100 ARR 986257) yielded *Campylobacter* inhibition zones of 10–13 mm dia. These studies show citrus-derived natural compounds have potential applications as inhibitory agents against both ciprofloxacin-sensitive and ciprofloxacin-resistant *C. jejuni* present on retail raw chicken carcasses.

P4-46 Antibacterial Efficacy of Thyme and Oregano Vapors

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Innovative applications of essential oils of spices may include their use as sources of antimicrobial volatile compounds without direct addition of the oil to the food system. The effect of thyme and oregano essential oil vapors dissolved in ethanol (5, 15, 25, 35 or 50%) on *Escherichia coli*, *Salmonella* Typhimurium and *Pseudomonas aeruginosa* growth response were evaluated. Thyme and oregano were milled and water vapor distilled to obtain their essential oils. For every microorganism, tripticase-soy agar plates were spiral inoculated (50 L of a 10^8 CFU/mL stationary phase bacterial suspension), incubated at 25°C in 5 liter hermetic chambers with 2 mL of essential oil/L of air, and observed every day for 5 days, registering and counting survivals. Increasing ethanol concentration to dissolve thyme or oregano essential oils significantly ($P < 0.05$) affected bacteria growth response. Bacterial count reductions could be attributed to a fast volatilization of volatile compounds. Minimal inhibitory concentration for every studied bacteria depends on the essential oil concentration, being higher for thyme essential oil. In general, *P. aeruginosa* was more resistant, followed by *E. coli* and *Salmonella* Typhimurium. An atmosphere with 2 mL of 25% essential oil/L air had an important effect on microbial growth being inhibitory for the three studied bacteria. Therefore, thyme and oregano essential oils volatiles are promising antibacterial agents for foods, where the essential oils have compatible flavors and odors.

P4-47 Antimicrobial Agents Combining Thymol, Carvacrol, and Eugenol

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Phenolic compounds naturally present in plants can be used as antimicrobial agents. The possibilities to formulate effective antimicrobial agent mixtures can be expanded by combining natural agents. Individual and combined effects of thymol (Th), carvacrol (Cr) and eugenol (Eu) concentrations on the growth of *Escherichia coli* O157:H7 (2 strains: 43895 and 932) and *Listeria monocytogenes* (2 strains: Scott A and 310) were evaluated. Tripticase soy broth was prepared with NaCl and hydrochloric acid or sodium hydroxide to reach a_w 0.99 and pH 7.0; and the necessary amount of Th, Cr and/or Eu (0, 12.5, 25, 50, 100, 200 and 250 ppm) was added; then samples were inoculated (10^6 cell/mL), and incubated at 5, 20 or 30°C (for *L. monocytogenes* strains) or at 20, 30 or 40°C (for *E. coli* strains), and optical density (OD) was measured every day for 7 days. Minimal inhibitory concentrations (MIC) for phenolic compounds (Th, Cr, and Eu) were determined, as well as the inhibitory concentrations for every antimicrobial combination. Fractional inhibitory concentrations (FIC) and FIC Index were then calculated. In general, *L. monocytogenes* exhibited higher resistance than *E. coli* to the evaluated antimicrobials. MICs varied from 250 to 12.5 ppm depending on strain and incubation temperature. Inhibitory concentrations of Th, Cr and Eu in the evaluated mixtures depended on strain and incubation temperature, but al least one synergistic combination was found. The combination of 25 (Cr), 100 (Th) and 50 (Eu) ppm inhibited growth of *L. monocytogenes* Scott A, while 25 (Cr), 100 (Th) and 12.5 (Eu) ppm inhibited *E. coli* O157:H7 (strain 43895). In several cases, FIC indexes evidenced synergic combinations with values < 0.7. Ternary mixtures of phenolic compounds reduced individual inhibitory concentrations. Combining agents from different sources may well increase the application of natural antimicrobials in foods.

P4-48 Antimicrobial Properties of Plant Extracts against *Clostridium perfringens* and *Vibrio cholerae*

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Introduction: The high incidence of diseases caused by foodborne pathogens requires the development of new and improved methods of food preservation. The food industry is trying to reduce the use of chemical preservatives in foods because of negative consumer perceptions of artificial preservatives, so as to either completely remove or adopt more "natural green" alternatives. Historically, plants have provided a good source of antimicrobials 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 such as *Clostridium perfringens* and *Vibrio cholerae*.

Purpose: In this study we intended to determine the inhibitory effect of different methanolic extracts against growth of two strains of *C. perfringens* and *V. cholerae*.

Methods: Microbial cultures were activated in Thioglycollate Broth (*C. perfringens*) and Luria Bertani Broth (*V. cholerae*) and incubated at 37°C overnight.

To obtain the plant extracts, 37 plants were dried and ground, then extracted overnight with methanol, filtered, evaporated under reduced pressure and resuspended in 10 ml of methanol. Preliminary antimicrobial assays were performed using the agar well diffusion bioassay in Mueller Hinton Agar plates. Minimum bactericidal concentrations (MBC) of the most active methanol extracts were determined using the broth dilution method; this concentration was the lowest concentration at which bacteria failed to grow in LB Agar (for *V. cholerae*) and Count Plate Agar (for *C. perfringens* under anaerobic conditions).

Results: The most bactericidal extracts for both bacteria were those of basil (*Ocimum basilicum L.*) 2–3 mg/ml, Japanese plum (*Prunus salicina Lindl.*) 2–6 mg/ml, sweet acacia (*Acacia farnesiana L.*) and white sage brush (*Artemisia ludoviciana Nutt.*) 0.3–0.5 mg/ml.

Significance: Here we present four plant extracts that could be alternatives to control growth of these pathogens. Basil and plum are edible plants that could be easily added to foods.

P4-49 Identification of Plant Compounds That Inactivate Shiga Toxin

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Introduction: Numerous foodborne diseases result from ingesting foods that are contaminated with bacterial toxins. The recent occurrence of foodborne-related outbreaks caused by Shiga-toxin producing *Escherichia coli* O157:H7 make it a public health problem of serious concern. Thus, in order to improve food safety, there is a need for technologies to inactivate or inhibit bacterial toxins.

Purpose: The specific objective was to develop a Vero cell-based assay to identify natural compounds from green tea and other plant compounds that inactivate Shiga toxin 2 (*stx*2) from enterohemorrhagic *Escherichia coli*.

Methods: Vero cell line (Vero-d2EGFP), which expresses a destabilized variant of the enhanced green fluorescent protein (EGFP), was used to monitor the inhibition of protein synthesis by *stx*2. Plant compounds and *stx*2 were added to the Vero-d2EGFP cells, and the EGFP fluorescence emitted by these cells was quantified after overnight incubation.

Results: Addition of the green tea compound, epillocatechin-3-gallate to *stx*2-treated cells resulted in 25% reduction when compared to toxin-untreated cells. In contrast, Vero-d2EGFP cells treated with *stx*2 had more than a 60% reduction of EGFP fluorescence. Interestingly, the essential oils oregano and cinnamon oil effectively inactivated *stx*2; EGFP production by these cells reached levels that were identical to those of toxin-untreated cells. Similar results were obtained for carvacrol and transcinna-maldehyde, the major constituents of oregano and cinnamon oil, respectively.

Significance: The proposed research has validated a novel method to identify anti-toxin compounds. In addition, the present study will provide basic information on the compound-induced inactivation of *stx*2 for the development of food-compatible conditions for toxin inactivation.

P4-50 Inhibitory Effect of Fermented Kimchi on *Vibrio parahaemolyticus* ATCC 17802 Strain

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The effect of kimchi, traditional Korean fermented vegetables containing diverse LAB, on inactivating *Vibrio* spp. and the factors affecting the antimicrobial activity of kimchi were investigated in this study. Commercial fermented kimchi containing 2–3% salt and pH between 4.2–4.4 inactivated more than 6 log CFU/g *Vibrio parahaemolyticus* ATCC 17802 after 10 min incubation at 37°C. Fermented kimchi of pH 4.3 inactivated *V. parahaemolyticus* ATCC 17802 more than the less fermented kimchi of pH 4.8 by 4 log CFU/g after 20 min incubation at 37°C. Of the raw ingredients in kimchi, including cabbage, spring onion, garlic, ginger and pepper, raw garlic showed the strongest antimicrobial activity against *V. parahaemolyticus* ATCC 17802. Less than 10% (w/v) garlic also accelerated the kimchi fermentation. The strong antimicrobial effect of fermented kimchi on *V. parahaemolyticus* ATCC 17802 is caused by low pH and the presence of lactic acid bacteria (LAB).

P4-51 Antimutagenic Capabilities of Selected *Lactobacillus* spp. and *Bifidobacterium bifidum* ATCC 11863 Supernatants at Different Growth Phases

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Introduction: Probiotic bacteria especially *Bifidobacterium* spp. and *Lactobacillus* spp. have been shown to be beneficial in human health. However, giving the difficulties of routine cultivation of anaerobic bacteria, direct administration of a live culture remains unreliable. An alternative approach would be to isolate the probiotic compounds and utilize them as food supplements thus avoiding the over reliance on probiotic bacterial colonization in the gut.

Purpose: The goal of this study was to evaluate the antimutagenic capabilities of *Bifidobacterium bifidum* ATCC 11863 and selected *Lactobacillus* spp. supernatants at different growth stages with the potential to identify and characterize functional probiotic compounds.

Methods: The *B. bifidum* and *Lactobacillus* spp. were grown in deMan Rogosa Sharpe (MRS) medium supplemented with 0.05% cysteine and incubated at 37°C in an anaerobic jar. Samples of mid-log and late stationary stage of each bacterial culture were centrifuged and supernatants were analyzed. A modified Ames test using *Salmonella* Typhimurium TA100 was performed to evaluate the antimutagenic activities of the bacterial supernatants against benzo[a]pyrene (0.5 mg/plate) and sodium azide (0.5 mg/plate).

Results: The bacterial supernatants were characterized with different patterns of antimutagenic activities against the two mutagens in the mid-log

and stationary phase. While *B. bifidum* ATCC11863 expressed higher antimutagenic activity against the benzo[a]pyrene in the mid-log (26.01 ± 12.7%) than in the stationary phase (9.54 ± 5.3%), all three *Lactobacillus* strains (ATCC8014, ATCC11578, and ATCC 4794) were antimutagenically active in the stationary phase (74.24 ± 15.8, 23.44 ± 14.8, and 49.10 ± 7.1% respectively) but not in the mid-log phase. Antimutagenic properties of the bacterial supernatants of the four cultures against the sodium azide were greater in the stationary phase (31.68 ± 8.7, 54.64 ± 8.1, 56.57 ± 13.1, and 43.58 ± 15.1%) than in the mid-log (26.01 ± 14.4, 44.42 ± 15.8, 29.25 ± 0.5, and 34.31 ± 12.6%).

Significance: The evaluation of the antimutagenic activities of the culture supernatants at different growth phases will facilitate the isolation and characterization of specific antimutagenic compounds.

P4-52 Management of Food Additives in Functional Health Foods in Korea

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Introduction: In Korea, current food additive regulation for functional health foods (HFF) is based on the Food Additives Code of Korea. However, the food additive regulation in Korea is different from that in other countries and from the international standard of food additives for HFF. Therefore, we needed to manage food additives for HFF that should be harmonized by international standard.

Purpose: The objective of this study was to provide a systematic administration plan for food additives used in HFF in Korea.

Methods: We analyzed the labeling of 95 HFF (capsule type: 18 domestic and 27 imported products, tablet type: 23 domestic and 27 imported products) marketed in Korea, and analyzed the opinion of food additives use for HFF produced in seven HFF manufacturers. Also, we detected preservatives (sorbic acid, benzoic acid, p-hydroxy benzoic acid and propyl paraben) in 11 HFF products which were similar to those rejected for imported ones.

Results: The identified use of food additives in 14 products (11 nutritional supplement products, 1 glucosamine product, and 1 gamma linolenic acid product) was not labeled, although HFF must have the label of the name and identified use of food additives currently listed in the Korea Food Additive Code. There were suggestions of limited residual mineral oil and legal use of three media ingredient additives as HFF manufacturer's opinion investigations. Also, perservatices were not detected in HFF products. Finally, the scientific information of 21 food additives selected from analysis of the label and manufacturer's opinion was listed mainly based on Korea Food Additives Code, CODEX and EU standard, JECFA and main developed countries' public reports and recent international papers in order to suggest the standards of 4 food additives (Food Blue No. 1, BHA, BHT, and Propyl Gallate).

Significance: The current results may be used as a reference to establish the revision of the standard. Moreover, further study will be accomplished monitoring of intake and exposure amounts of food additives for the safety assessment of HFF.

P4-53 Determination of Arsenic, Lead and Manganese in Water Samples from Hidalgo and Tlaxcala, Mexico

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Introduction: The toxic effects of heavy metals is a widespread concern related to reduction of environmental pollution in the modern industrial context. Virtually all metals can produce toxicity when ingested in sufficient quantities, but there are several which are especially important because they either are so pervasive, or produce toxicity at such low concentrations.

Purpose: The purpose of this study was to determine the presence of of arsenic, lead and manganese in water samples from Hidalgo and Tlaxcala, Mexico Methodology: 330 samples of water obtained at Hidalgo and 104 from Tlaxcala during January to December of 2006 were analyzed by atomic absorption spectroscopy with Analyst 800 spectrophotometer. 417 samples were analyzed for arsenic and lead, and 17 for manganese only. The samples were obtained from public supply (potable water) or natural sources such as lakes and rivers; 10 were purified water.

Results and Conclusions: The results obtained were compared with the maximal concentrations established in the Mexican Laws, 0.025 mg/l for arsenic and lead and 0.15 mg/l for manganese. Of 417 samples analyzed for arsenic and lead, 51 (12.23%) were positive for the presence of arsenic with a maximum concentration of 0.24 mg/L, and 4 (0.95%) for lead at a concentration of 0.24 mg/L. Of the 17 samples analyzed for manganese, only one had a concentration of 0.372 mg/L. The results obtained by the laboratory are analyzed by the Jurisdictions and the State Commission of Protection against Sanitary Risks who take the necessary measurements to avoid poisonings for the consumption of water contaminated with heavy metals.

P4-54 Monitoring of Ochratoxin A in Imported Foodstuffs

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Introduction: Ochratoxin A produced by several *Aspergillus* and *Penicillium* species has carcinogenic, immunosuppressive, nephotoxic, and teratogenic properties. Recently, Ochratoxin A has been analyzed

in distributed foodstuffs such as dried fruits, grains, etc., and the frequency of detection was rare in Korea. It is necessary to verify the safety of imported foodstuffs with regard to Ochratoxin A.

Purpose: The purpose of this study is to monitor the levels of Ochratoxin A comtamination in imported foodstuffs.

Methods: Sample (25 g) pulverized and blended was extracted with solvent (acetonitrile: water = 6:4) for 3 min. The extract was purified with Immunoaffinity column and analyzed by HPLC. The analyte (10 l) was injected onto a C18 column (4.6 x 250 mm, 5 m, Shiseido capcell pak MGII) and detected with a fluorescence detector (excitation wavelength 333 nm, emission wavelength 460 nm). The mobile phase consisted of methanol, water, acetonitrile (99:99:2).

Results: The 56 samples of imported foodstuffs such as dried grape, rice, barley, and fruit product were analyzed for Ochratoxin A. Among the samples analyzed, only one sample, dried grape, proved to be contaminated, at the level of 0.2 g/kg.

Significance: The detection level was less than the potentially recommended standard, 10 g/kg, of Ochra toxin A in Korea. However, Ochratoxin A can be a potential emerging hazard factor because grains and grain products are the most widely consumed foodstuffs in Asia. The concentration of detected Ochratoxin A was 0.2 g/kg, and this suggested that use of IAC improved the purification of sample.

P4-55 Safety Evaluation of *Elsholtzia splendens* Extracts: Assessment of Acute Toxicity and Mutagenicity

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Introduction: Much attention has been gained recently for *Elsholtzia splendens* extracts and an issue on their usage is raised due to their healthful properties. However, there is no sufficient background information on toxicological evaluation of *Elsholtzia splendens* extracts to give an assurance of safety for developing dietary supplements and functional foods.

Purpose: The objective of this study was to evaluate safety of *Elsholtzia splendens* extracts, using tests for acute oral toxicity, bacterial reverse mutation, and chromosome aberration.

Methods: Both female and male mice were orally administrated *Elsholtzia splendens* extracts at the concentrations of 0, 500, 1000, and 2000 mg/kg body weight/day. Mutagenicity of the extracts was evaluated in a bacterial reverse mutation assay, using histidine-requiring *Salmonella* Typhimurium (TA 98, TA 100, TA 1535, and TA 1537) and tryptophan-requiring *Escherichia coli* (WP2uvrA). In vitro chromosome aberration assay in Chinese Hamster Lung (CHL) was conducted to evaluate genotoxicity.

Results: Single administration of dose levels of 500, 1000, and 2000 mg/kg body weight/day to mice for 15 days did not produce any significant mortality, clinical signs, body weight loss, or changes in gross findings. *Elsholtzia splendens* extracts in the range of 156.3–5000 ug/plate did not induce mutagenicity in *S.* Typhimurium and *E. coli* with and without metabolic activation system. Any significant chromosomal aberration was not observed in CHL cells 6 h after treating with the extract at the concentrations of 1250, 2500, and 5000 g /ml in the absence or presence of metabolic activation system. However, frequency of chromosomal aberration in 22 h after treatment without metabolic activation system was increased, showing a pattern of dose-response relationship. The highest concentration of 5000 g/ml significantly induced chromosomal aberration.

Significance: Elsholtzia splendens extracts may induce chromosomal structure abnormality in CHL cells. This study suggests that further study is needed to assess the potential genotoxic effects of *Elsholtzia splendens* extracts.

P4-56 Acrylamide Content in Food Products Manufactured in Korea

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Acrylamide is a toxic and probably cancer-causing, industrial chemical used in the preparation of industrial polyacrylamide, which is used in a variety of industries such as wastewater treatment and paper and pulp processing. Acrylamide is not novel, but its occurrence in food raises concern about the human health affects of acrylamide intake. Acrylamide is found in many common food products, in particular carbohydrate-rich foods such as potatoes cooked at high temperature. The purpose of this study is to analyse acrylamide in food products consumed in Korea and to estimate intake of acrylamide from food products manufactured in Korea. Around four-hundred seventy food products, categorized into seventeen groups, were purchased at local markets and analyzed for the acrylamide concentration with an LC-MS/MS method. Samples were selected based on special consideration such as expert consultation, Korean food consumption data and food market shares that were considered representative. The acrylamide content of Korean food products is similar to that reported for domestic and international analyses. For 471 food products, acrylamide content ranged from ND (not detected) to 4,002 g/kg. Acrylamide levels in cereal, coffee, potato snacks, chocolate, and prune juice are relatively high.

P4-57 A Limited Survey of Zearalenone, an Estrogenic Mycotoxin, in Cereal Crops and Their Products Consumed in Korea

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Zearalenone is an estrogenic mycotoxin produced by a variety of Fusarium fungi, which are contaminants of cereal crops worldwide. It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperestro-genic syndromes in humans. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a

provisional maximum tolerable daily intake (PMTDI) for zearalenone of 0.5 g/kg of bw/day. In this study, zearalenone contamination levels were analyzed in selected cereal crops and their products consumed in Korea, and dietary intake of zearalenone was estimated as a preliminary exposure assessment. The total of 70 samples (14 of polished rice, 14 of barley, 14 of corn, 7 of unpolished rice, 7 of wheat flour, 7 of sweet corn and 7 of breakfast cereals) were collected according to a stratified random sampling design and zearalenone levels were analyzed with HPLC with fluorescence detection (limit of detection; 3.1 ng/g, limit of quantification; 10.1 ng/g). Dietary exposure to zearalenone was assessed for the population groups of different ages and genders by use of our contamination data and based on a Monte-Carlo simulation. Twenty-four percent of samples (17/70) were contaminated with zearalenone. Zearalenone was detected in unpolished rice, barley and dried corn, with the average contaminations of 23.4, 7.7 and 29.5 ng/g, respectively. The highest contamination level was 174.9 ng/g in dried corn. The estimated average daily exposures to zearalenone for male population groups ranged from 0.3 to 2.7 ng/kg bw/day. The estimated 95th percentile exposure for the 3 to 6 year-old male group, the most vulnerable group to zearalenone exposure, was 7.8 ng/kg bw/day, which corresponds to a PMTDI of 1.56%. The estimated exposures to zearalenone far below the % PMTDI recommended by JECFA suggested that Korean populations are not under the risk of zearalenone exposure.

P4-58 Fluorescence Polarization-based Homogeneous Assay for Zearalenone Determination in Grains

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Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin commonly found in cereal grains. Rapid determination of ZEN is required to monitor and divert effectively contaminated grains from the food supply. In this study, a fluorescence polarization immunoassay (FPIA) was developed and compared with enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) for the determination of ZEN. FPIA, based upon the competition of unlabeled ZEN from a grain sample with a fluoresce in-labeled ZEN for a ZEN-specific antibody was easer to perform and quicker (< 2 min per sample) but less sensitive than ELISA and HPLC (a detection limit of 0.014 g/mL). Recoveries from spiked maize averaged 106.4 ± 12.5% for FPIA, 107.4 ± 15.9% for ELISA and 105.4 ± 10.7% for HPLC. Seventy samples of field-contaminated grains (maize, barley, rice, wheat, and their products) were evaluated by FPIA, ELISA and HPLC methods, and the results of FPIA in grain samples correlated well with those of HPLC and ELISA ($r^2 = 0.8 - 0.9$, $P < 0.05$). The reliability of FPIA, which was defined as the deviation from the true values for certified reference material, was comparable to those obtained with ELISA and HPLC. These results suggest that FPIA has substantial potential as a screening tool for ZEN in grains without complicated clean up

P4-59 Development of Immunoassay for the Detection of Zearalenone

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Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* species. It is found worldwide in a number of cereal crops such as corn, barley, rice, wheat and oats. A direct competitive enzyme-linked immunosorbent assay (DC-ELISA) and a rapid immunochromatographic assay (ICA) based on the monoclonal antibody (MAb) were developed for determination of the ZEA. The cross reactivates to other mycotoxins of MAbs against ZEA were -zearalenol, -zearalenol, -zearalanol and -zearalanol as 121%, 65%, 21% and 19%, respectively. The detection limit of ZEA was determined to be 0.35 ± 0.07 ng mL^{-1} and IC_{50} was equal to 2.34 ± 0.28 ng mL^{-1} analyzing by the DC-ELISA. The detection limit in the ICA was 1.00 ng mL^{-1} and required assay time lower than 15 min. The extraction method with methanol-water (80:20, v/v) gave higher extraction efficiency as percentages of recoveries ranging 90.65 –116.54% for the ZEA determination in rice, barley and corn. Matrix effects can be avoided when a 1:9 dilution of rice, barley and corn samples in phosphate-buffered saline is used. The detection limit of the ZEA for rice, barley and corn were 0.42, 0.63 and 0.54 ng mL^{-1}, respectively by DC-ELISA and 2.5 ng mL^{-1} by ICA. All 80 rice and 50 barley samples were considered to be negative by using ELISA and ICA test strip. However, among 38 corn samples, ZEA was detected in 9 and 5 samples by DC-ELISA and ICA, respectively. The positive samples of DC-ELISA and ICA were confirmed by HPLC, and 7 samples were detected by HPLC. The data of ZEA determination in corn showed very good agreement between DC-ELISA, ICA and HPLC. Development of DC-ELISA and ICA are useful for screening of ZEA in rice, barley and corn samples.

P4-60 Construction and Expression of a Synthetic Single-chain Variable Domain Fragment Antibody against Mycotoxin Zearalenone in *Pichia pastoris*

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Zearalenone [6-(10-hydroxy-6-oxo-trans-1-undecenyl) -resorcyclic acid lactone, ZEN] is a mycotoxin produced by members of the genus Fusarium after infection of foods and feedstuffs. A synthetic gene coding for a single-chain variable domain fragment antibody (scFv-ZEN) reactive with ZEN has been

designed, constructed, and expressed in *Pichia pastoris*. The nucleotide sequence for expression in *Pichia pastoris* was optimized to explore the influence of codon usage on expression as compared to the native scFv-ZEN open reading frame (ORF). Three successive rounds of PCR using 12 oligonucleotides were used to generate a synthetic scFv-ZEN ORF, which consists of variable region of the heavy chain (VH) and light chain (VL) with a flexible linker, optimized for codon usage. Expression levels were examined in small-scale shaker culture by use of a defined medium. Comparable levels of expression from the native and synthetic scFv-ZEN constructs were observed. Minor differences in scFv-ZEN expression were found at various culture temperatures and pH values used. No detectable protease activity was observed, and the expression level was stable over 96 h. Expressed scFv-ZEN was detected by western blot analysis and its biological activity was demonstrated by surface plasmon resonance analysis. Our results demonstrate the potential of soluble scFv-ZEN for developing a rapid and affordable immunoassay for detection of ZEN in foods and feedstuffs.

P4-61 Thermostable Antigenic Proteins in Fish

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Introduction: Fish are listed as one of the "big-eight" major food allergens. The Food Allergen Labeling and Consumer Protection Act requires these allergenic foods or any protein-containing ingredient derived from these foods to be declared in the ingredient statement. Currently no rapid immunoassays are available to detect fish allergens in packaged foods to enforce the regulation.

Purpose: The objective of this study was to identify thermostable antigenic proteins in commercially important fish for the development of a suitable immunoassay.

Methods: Soluble proteins from raw, cooked (100°C for 20 min) and autoclaved (133°C for 20 min) fish were extracted from thirty different species of common food fish. Western blot was performed to reveal the antigenic proteins in fish extracts by using five cross-reactive monoclonal antibodies (2G8, 3E1, 3E4, 3E8, and 8A10) that were previously produced against heated catfish crude proteins.

Results: At least three major groups of proteins remained soluble after heat treatment of the fish and they were antigenic to these five MAbs tested. The first group consists of protein bands with high molecular weights, ranging between 100 and 150 kD, probed by MAbs 3E8 and 8A10 in fish extracts; the second group are proteins with a medium size, around 24 kD, probed by MAbs 2G8 and 3E4; and the third group of proteins that bind to MAb 3E1 are of a low molecular weight, between 11 and 13 kD. Multiple minor bands that appeared in each protein group might be heat degraded peptides of the same antigenic proteins. These three proteins appeared to be collagen, troponin I and parvalbumin, respectively.

Significance: Identifying suitable antigens in target fish is a prerequisite for antibody development. Once antigens are identified, it will be possible to develop specific antibodies against the isolated antigen for future development of immunoassays to detect different fish allergens in processed food.

P4-62 Effect of Heat Treatment on the Quantitation of Peanut Allergens by ELISA Test Kits

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Commercial ELISA tests are increasingly used by food manufacturers to validate allergen control measures. Quantitation in these tests is generally achieved via measurement of the antibody-antigen reactions; thus any changes in the antigenic property of the target proteins may greatly influence assay results. Heat treatment often leads to changes in the solubility and immuno-reactivity of proteins. This study examined how thermal processing affects allergen quantitation by ELISA kits. The performance of two types of ELISA tests (one that is reactive to total proteins and the other reactive to specific marker proteins) for quantitation of allergens in foods that have been subjected to either moist- or dry-heat treatments was examined. Defatted flour prepared from raw peanuts was heated in the presence of water at 60°C, boiled or autoclaved for 10 min or dry-heated at 60–400°C for 10 min. Commercial preparations of light-, medium- and dark-roasted peanut flour were also used. The amount of protein in the lab-prepared and commercial samples was measured, using the BCA total protein assay as well as two commercial ELISA kits. It was found that elevated heat treatment resulted in a lower level of protein extracted. Boiling and autoclaving caused an approx. 50% decrease in the amount of protein extracted as determined by the BCA assay. The amount of residual proteins in the autoclaved samples, however, was not accurately indicated by the test kits. The Biokits® Peanut Assay Kit, which employs antibodies specific to a heat labile protein (Ara h 1) underestimated the amount of residual protein by as much as 400 fold, although the extent of deviation was less with the Veratox® Quantitative Peanut Allergen Test. Similarly, both test kits greatly underestimated the amount of proteins in the dark-roasted peanut flour samples by 20–2000 fold. These results suggest that commercial test kits may not be able to accurately determine the amount of proteins present in thermally processed foods.

P4-63 Development of Real-time PCR Method to Monitor the Presence of Egg Allergens in Pasta Declared as Egg-free

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Introduction: Food allergies are a serious problem for the human population. People suffering from food allergies are dependent on accurate food labelling to prevent the allergic reaction. Even small amounts of food allergens can be dangerous for highly sensitized persons, so sensitive and reliable methods to monitor the correct declaration are needed.

Purpose: The purpose of this study was to develop a real-time PCR method for the detection of chicken eggs in pasta.

Methods: The real-time PCR method was developed, with a detection limit of 0.5% added chicken eggs in noodles or pasta. The primer pairs and probe for Real-time-PCR were designed for the region of chicken mitochondrial ATPase, subunits 8 and 6, and the size of the amplification product was 113 bp. The designed primers and probe yielded only chicken egg-specific amplification product.

Results: Twenty pasta and noodle samples from the market, declared as egg-free were tested by this method. In two cases, chicken eggs were detected, in contrary to the declared composition. This method is able to detect the addition of whole eggs or yolks only. No PCR signals associated with separated egg white were obtained.

Significance: This real-time PCR method is specific enough to detect whole chicken eggs in noodles or pasta from the market. This method can help Food Control Authorities in allergy prevention and control.

P4-64 Screening Procedures for Clenbuterol Residue Determination in Raw Swine Livers Using Lateral-flow Assay and Enzyme-linked Immunosorbent Assay

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Introduction: Clenbuterol, which can promote animal growth with increased accretion of skeletal muscle mass and decreased accretion of body fat, may cause symptoms of increased heart rate, muscular tremors, headache, nausea, and muscular cramps in patients. Due to the potential hazard for human health, use of clenbuterol has been prohibited in many countries, including EU, USA and China. It is essential to have an inexpensive, sensitive, convenient and reliable technique to screen for the presence of the heat stable (172°C) clenbuterol.

Purpose: The purpose of this study was to compare a rapid lateral-flow strip assay developed in our lab with a commercial ELISA kit (r-biopharm GmbH, Germany) for screening of clenbuterol in raw swine liver.

Methods: A total of 128 swine livers acquired from five local markets in Nanchang, China were tested. A 10 g portion of minced swine liver was extracted with ethyl acetate-isopropanol and perchloric acid and then cleaned up by solid phase extraction. The dry residues were dissolved in 2 ml of phosphate buffer pH 7.4 for analysis by lateral-flow strip and ELISA kit.

Results: Analysis was completed in 10 min with the lateral flow strip and in 90 min with ELISA. The detection limit of the strip was 1 ppb of clenbuterol. In parallel with ELISA, the rapid detection strip had no false negatives but had a false positive rate of 6.5%. Cross reactivity of the strip was assessed and found negative after tests with clenbuterol analogues such as tulobuterol, terbutaline, salbutamol, ractopamine, ritodrine and fenoterol.

Significance: These data suggest that a lateral-flow strip can safely be used as a screening method for a clenbuterol residue surveillance program and has great applied value in the food safety field, especially in developing countries.

P4-65 DSC Characterization of a Monoclonal Antibody Specific to Ruminant Blood

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Introduction: Ruminant plasma proteins are widely used as protein supplements in feed and food products. Because the spread of bovine spongiform encephalopathy (BSE) has been attributed to the transmission of the infective agent, a prion, though recycled ruminant animal by-products in feed, the European Union has prohibited the feeding of any animal proteins to farm animals. Currently no rapid methods are available for the detection of ruminant blood in heat-processed food or feed products.

Purpose: This study was done to characterize a monoclonal antibody (MAb), raised against bovine plasma proteins, for the potential development of an immunoassay for the detection of ruminant blood.

Methods: MAb 1H9 was developed against crude soluble bovine plasma proteins extracted from sterilized bovine blood. The specificity of MAb 1H9 was examined against raw, cooked (100°C, 15 min) and autoclaved (121°C, 15 min) blood samples from different species (bovine, ovine, rabbit, horse, chicken, porcine and turkey) and non-blood samples (bovine serum albumin, non-fat dry milk, soy protein, gelatin, egg albumin, and meat proteins) using indirect enzyme-linked immunosorbent assay (ELISA). The antigenic protein was identified by immunoblotting the blood extracts with MAb 1H9. The immunoreactivity of this antibody was also examined against various blood samples using a competitive ELISA.

Results: MAb 1H9 (IgG1) was specific to both bovine and ovine blood without cross-reaction against blood from non-ruminants and non-blood protein samples tested. The MAb recognized a 10 kD thermostable protein in fresh and heat-treated ruminant blood extracts. The reaction stability of this antibody was confirmed in both indirect ELISA and competitive ELISA, where antigens are present in either immobilized form or free form.

Significance: Results suggested that MAb 1H9 can be used to develop a rapid immunoassay for the detection of undeclared ruminant blood proteins in food and feed products, regardless of heat processing.

P4-66 Simultaneous Determination of Three Macrolide Antibiotics in Foodstuffs by High-performance Liquid Chromatography

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Introduction: Macrolide antibiotics are antibacterial agents widely used as veterinary drugs in food-producing animals to prevent disease and promote growth. However, residues of these antibiotics in

foodstuffs can lead to allergies or bacterial resistance. A simple and rapid analytical method to determine macrolide antibiotic residues in food is needed.

Purpose: The purpose of this study was to develop an analytical method to simultaneously detect three macrolides (spiramycin, tylosin and tilmicosin) by modifying pre-treatment and determination conditions, using high-performance liquid chromatography.

Methods: The residuary macrolide antibiotics in farm and marine products were extracted by acetonitrile and were analyzed by HPLC with photo diode array. The percentage recoveries of the modified method were evaluated by comparing spiked samples with standard solutions.

Results: When liquid extraction was applied to sample preparation, recovery percentage was higher than with solid phase extraction (spiramycin 83.5%, tilmicosin 89.0% and tylosin 84.4%). Acceptable resolution was achieved by using HPLC conditions as follows; flow rate -1 mL/min, detection wavelength -232 nm (spiramycin) and 288 nm (tilmicosin, tylosin), mobile phase : solution A-0.025M phosphate buffer (pH 2.5), solution B-0.025M phosphate buffer (pH 2.5)/aceotnitrile (6/4), and gradient condition: 0(sol B 40%) -20 min (sol B 100%) -25 min (sol B 100%) -40 min (sol B 40%). The limits of detection (LOD) for fortilmicosin, tylosin, and spiramycin were 12.74, 12.78, and 7.34 ppb, respectively.

Significance: The analytical method modified in this study could produce accurate data for determining residue concentration of macrolides. In addition, it could be applied in farm and marine products at once.

P4-67 Comparison of Visual Inspection, an Allergen-specific Method (ELISA) and a Nonspecific Methods (Sensitive ATP and Total Protein) to Detect the Presence of Allergenic Food Residues on Food-contact Surfaces

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Introduction: Recent FDA and IFT surveys found that the most common method used by food manufacturers to check the effectiveness of their equipment cleaning program is visual inspection, followed by ELISA and bioluminescence/ATP, and other tests.

Purpose: The purpose of this study was to compare visual inspection, ELISA, and ATP sensitive swabs, and total protein swabs for detecting the presence of allergenic food residues on food-contact surfaces.

Methods: One ml of milk, egg and peanut solutions (0–1000 μg/mL) were pipetted onto the surface of stainless steel, urethane and Teflon plates. The plates were dried in an oven (80°C) to obtain 0–1000 μg of the food residues. The plates were visually examined and then simultaneously swabbed for ELISA, sensitive ATP, and total protein testing.

Results: In general, the detection limits (DL) for visual inspection were consistent with those determined by ELISA. The DL (visual, ELISA and ATP) for egg was 500 μg on all food contact surfaces, but lower with the total protein swabs. The ELISA and visual detection limit for peanut was 500 μg on all food surfaces, but 50–100 μg when tested with the ATP and total protein swabs. ELISA and visual tests detected milk at 100 μg on all food surfaces, but 10–50 μg when tested by sensitive ATP swab and total protein swabs. In some cases, total protein swabs detected food residues at or below levels detected by sensitive ATP swabs.

Significance: This study shows that limitations for these methods exist and care must be taken when choosing a method for detecting the presence of food residues on food-contact surfaces.

P4-68 A High-sensitivity Immunoassay for the Detection of Ruminant Muscle Protein in Meat and Bone Meal and Feeds

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Introduction: Determination of the presence of ruminant content in feeds is essential to limit the spread of BSE. Regulations in both Europe and the United States require that there be no ruminant proteins in ruminant animal feeds (with exceptions for blood, milk and gelatin). To enforce this ban, the FDA has suggested the use of quick test kits or microscopy at all facilities that manufacture ruminant feed.

Purpose: The purpose of this assay is to provide quick, reliable screening of ruminant feeds for the presence of ruminant proteins prior to sale or export.

Methods: While microscopy has been the standard method for the detection of ruminant content in feeds, it is costly, time-consuming, and non-species specific, as well as requiring extensive analyst training. Similar issues make NIRM, NIRS and PCR undesirable as screening assays. The MELISA-Tek Ruminant Assay is an ELISA method that detects troponin-I, a heat-stable, muscle-specific protein. Thus it detects the major banned ruminant protein (muscle) after high-temperature processing, without detecting exempted materials. A concentration protocol has been developed that makes the MELISA-Tek Ruminant assay a sensitive screening tool.

Results: Slaughtering processes typically remove the majority of muscle tissue from carcasses, leaving little muscle tissue when rendered. Nonetheless, as originally developed, the MELISA-Tek Ruminant assay had a limit of detection (LOD) of 0.005% for ruminant muscle tissue and 0.5–1% for ruminant meat and bone meal (MBM), in feed. The high-sensitivity protocol improves the LOD to 0.1% (w/w) for commercial beef and sheep MBM in feeds without increasing its response to feed matrices or non-ruminant tissues.

Significance: This protocol should increase the utility of ELISA assays in screening ruminant feeds for sale or export, and help guarantee the safety of the ruminant feed supply.

P4-69 Withdrawn

P4-70 Rapid Detection of Potential Food Allergen Residues on Processing Equipment – a Tool for Confirming Effective Cleaning

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Introduction: Food allergens are increasingly being recognized as a serious health issue, with an estimated 5–8% of North American children allergic to one or more foods. Food manufacturers often produce allergen-containing foods and allergen-free foods on the same manufacturing equipment. As such, it is very important that manufacturers ensure that cleaning procedures have been effective in removing any residual protein.

Purpose: The purpose of this study was to evaluate the effectiveness of a rapid swab test for the detection of residual protein when challenged with food allergen in solution or dried onto surfaces.

Methods: Samples of allergenic food (egg, milk, peanut, cereal gluten, soy, tree nut and buckwheat) were used to determine the limit of sensitivity of the Aller-tect swab for each food ingredient. At each level tested, 100 µl solution of sample was applied directly to the swab, and the test was performed as per instructions. The recovery from various surfaces (stainless steel, plastic and glass) was determined as follows: 40 and 100 µg of egg, milk and peanut standards were each delivered to 5 x 5 cm surfaces, spread and dried. The swab was then used to assess protein recovery from the surface.

Results: The swab test provided clear visual results within 15 – 25 min. All food allergens tested were detected at less than 40 µg protein in the reference materials (egg and milk ~ 10–20 µg; soy, almond and buckwheat ~ 20–40 µg; gluten < 40 µg; peanut ~ 25–50 µg). All the 100 µg samples were recovered from all surfaces, while the 40 µg egg and milk samples were recovered from glass and plastic surfaces.

Significance: Results showed that this swab test is simple to use, can detect very low levels of protein, and is suitable for assessing the presence of potential food allergens on a range of surfaces in food production environments.

P4-71 Recovery of Staphylococcal Enterotoxin in Multiple Phase Foods

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In multiple phase foods, the water soluble protein staphylococcal enterotoxin becomes trapped in the water molecules contained in the lipid fraction of such foods. This phenomenon has an influence on maximizing the recovery of enterotoxin from multiphase foods. Also, the amount of lipid in foods will determine the difficulty of removing the enterotoxin. As a consequence, partitioning studies were conducted, using a toxin insoluble organic solvent ($CHCl_3$) to relieve the constraints produced by the lipid fraction in the removal of toxins from this class of food product. Enterotoxin serotypes A, B and D were added at toxin concentrations of 2 ng/ml to five brands each of whole milk, 1% low fat milk, half and half, eggs and two kinds of salad dressings (Italian and ranch). Prepartition and postpartition extracts from the foods were assayed for toxin recovery, using an ELISA-based serological method in a double antibody sandwich configuration. While most of the toxin was, generally, recovered by traditional extraction procedures involving homogenization of the sample after addition of a buffer (post partition) and high speed centrifugation to yield an extract, the multiphase foods investigated retained some of the enterotoxin in the lipid fraction of the food products. Re-extraction of the lipid fractions with the organic solvent was required to remove residual toxin trapped in the water molecules of the lipid portions of the products. These studies showed that the enterotoxins (A [0.2-19.8%], B [0.11-22%], and D [0.1-4.2%]) retained in the lipid fractions were released after solvent extractions. The difficulty in the removal of enterotoxin (the number of lipid extractions) from these lipid fractions was influenced by the complexity and amount of lipid in the food products. Although traditional extractions should suffice in low fat containing foods, partitioning would be advised for high fat containing foods or where there exist very small traces of enterotoxin in multiphase foods.

P4-72 DSC Ethylene Production by *Penicillium expansum* and Its Endogenous and Exogenous Effects on Patulin Production in Inoculated Solid Media

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Introduction: Patulin is produced by mold species such as *Penicillium expansum* and is the most abundant mycotoxin in apples and apple juice. Production of patulin in infected apples can be reduced by modified atmosphere fruit storage conditions.

Purpose: The purposes of this research were to determine the production of ethylene and patulin by *P. expansum* in apples and solid media and the influence of exogenous ethylene and 1-MCP on patulin production by *P. expansum* when cultured on these media.

Methods: Spores of *P. expansum* ATCC 28883 and/or ATCC 1117 were cultured in tubes containing PDA or PDB. Timing of patulin and ethylene production by *P. expansum* was inversely related for both strains. Other experiments were conducted on plates containing PDA or PDB and Red Delicious apples treated with *P. expansum* ATCC 28883 in a flow-through system with combinations of ethylene and 1-MCP using a factorial design. Samples from each container were obtained every two days after treatment. Patulin in apple juice was quantified using solid phase extraction followed by high performance liquid chromatography.

Results: Patulin production in-petri plates containing PDA inoculated with *P. expansum* ATCC 28883 were significantly increased by the presence of exo-

genous ethylene (= 0.0206). There was no overall 1-MCP effect (= 0.72) on patulin production. Concentrations of patulin in apple juice of *P. expansum* inoculated apples increased exponentially from undetectable (< 4 μg/L) concentrations at day 0 to 3.16 ± 0.10 g/L after 16 days of storage, respectively ($P \leq 0.0182$). Averaged across all time points, exposure of apples to 100 μL/L ethylene tended ($P \leq 0.065$) to increase the production of patulin in *P. expansum* inoculated apples.

Significance: These results confirm that *P. expansum* produces ethylene and suggests an inverse relationship between ethylene and patulin production. We conclude that, contrary to our original hypothesis, exogenous ethylene treatment does not inhibit (and may actually promote) patulin synthesis by *P. expansum*.

P4-73 Reduction of the Allergenicity of Hypoallergenic Formula by Gamma Irradiation

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Introduction: Cow's milk allergy is the most common food allergy during infancy and early childhood. However, hypoallergenic formula for infants can induce allergic disease.

Purpose: This study was conducted to reduce allergenicity of hypoallergenic formula by using gamma irradiation.

Methods: After extraction of soluble protein from hypoallergenic formula (HAF) and gamma-irradiated hypoallergenic formula (GIHAF), SDS-PAGE, immunoblotting for milk allergic human IgE and ELSIA with human IgE for ßlactoglobulin (BLG) were analyzed.

Results: The results of SDS-PAGE and immunoblotting with human IgE did not show any band in HAF and GIHAF. In the results of ELISA, allergenicity of HAF was detected with human IgE for BLG; however, that of GIHAF was not detected.

Significance: Gamma irradiation could be of use for the reduction of allergenicity of hypo-allergenic formula. Further studies are mandatory to develop hypoallergenic dairy products.

P4-74 Thermal Stability of Ricin in Apple Juice

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Introduction: Ricin is a potent protein toxin found in the seeds of the castor bean plant, *Ricinus communis*. Ricin specifically and irreversibly inactivates ribosomes, promoting cell death by inhibiting protein synthesis. Although several reports indicate that ricin can be heat detoxified, the conditions required for inactivation are not well characterized.

Purpose: The objective of this work was to determine the effects of heat treatments on the detection and toxicity of ricin added to apple juice.

Methods: Two different brands of single-strength apple juice containing 100 μg ricin/mL were heated at 60–90°C for up to 2 h. The heat-treated juices were analyzed by ELISA to determine levels of detectable ricin. The residual cytotoxicity of ricin-containing juice after heat treatments was determined in an anchorage-dependent transformed macrophage cell line (RAW264.7 macrophage cells).

Results: The ELISA and the cytotoxicity assay indicated that ricin detection and toxicity decreased with increasing heating times and temperatures. The half-lives of ricin, determined by ELISA, in one brand of apple juice at 60, 70, 75, 80, 85 and 90°C were >200 min, 26 min, 4.2 min, 3.3 min, 1.5 min, and 0.96 min, respectively. Similar half-lives were obtained by ELISA detection of ricin in the second apple juice brand. The half-lives for ricin cytotoxicity in both juices were similar to those found for ELISA detection.

Significance: The results indicate that ricin is a relatively heat stable protein in apple juice and may remain toxic under some thermal processing conditions.

P4-75 Reduction of Aflatoxin in Rice and Corn by Different Cooking Methods

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Introduction: Aflatoxin is a secondary fungal metabolite and is a health hazard because it is a human carcinogen and has many deleterious effects in humans and animals. Corn and rice are substrates for the fungus which can produce aflatoxin,

Purpose: The purpose of this study was to investigate aflatoxin reduction resulting from the cooking of rice and corn, and to compare the reduction rate under different cooking conditions.

Methods: Aflatoxin was produced by *Aspergillus parasiticus* ATCC 15517 on a type of brown rice and a corn. Several kinds of cooking methods (washing, steaming, baking, popping boiling and/or fermentation) were used to reduce aflatoxin in the rice and corn. The aflatoxin content in the samples was determined by high performance liquid chromatography with a fluorescence detector.

Results: The total aflatoxin levels of the rice and corn (27.46 ppm and 43.80 ppm, respectively) were decreased, with no significant difference cause by washing with water. After cooking the rice and corn and averaging the total aflatoxin levels in the final products (cooked rice or corn bread, rice/corn cake, popped rice or popcorn, rice/corn gruel, and fermented rice beverage), the greatest reduction was found in the rice gruel (96.3%) and in the corn gruel (95.0%). Their total aflatoxin levels were decreased to 1.02 ± 0.54 ppm and 2.19 ± 0.99 ppm, respectively ($P < 0.05$).

Significance: The results of this study indicate that boiling, fermentation, popping, steaming, and baking were helpful in reducing the aflatoxin levels in the rice and corn. This study suggests that the most effective treatments for the rice and corn might be boiling and exposure to heat.

P5-01 DSC Extraction of *Bacillus anthracis* Spores from Milk

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The lack of effective pre-analytical methods for quick recovery of select agents in foods is a major weakness of our national food security system. Milk and other liquid foods that have short turnover times and large distribution areas are potential targets for bio-terrorist attack with a number of select agents, including *Bacillus anthracis*. The purpose of this study was to investigate methods of extracting and concentrating *Bacillus anthracis* from milk on the basis of hydrophobicity and density differences. Raw milk inoculated with specific levels of *B. anthracis* spores was subjected to heat, centrifuge treatments, and hexadecane to determine concentration efficiency. Inoculated milk was also centrifuged with a silicone oil layer with greater density than milk. Following centrifugation, spores were recovered from the pellet with peptone water and plated on tryptic soy agar to determine the rates of recovery. Spores of all three strains were effectively concentrated and extracted into a pellet (> 90% recovery of original inoculum) from inoculated raw milk that was first heated in an 85°C water bath for 60 s and then centrifuged with silicone oil. Optimal centrifugation speed and time were determined to be 4709 x g for 2 min. Very few spores were recoverable from milk that had not being heated prior to centrifugation, and milk heated for less then 60 s in an 85°C water bath resulted in lower recovery into the pellet. Mixing with hexadecane caused a migration of less than 5% from the milk phase. More than 70% of the spores were recovered from the cream. This data suggest that it is possible to rapidly and effectively concentrate and extract spores from milk. Detecting *B. anthracis* contamination before the milk enters the distribution chain would prevent consumption and illness.

P5-02 DSC Addressing Biosecurity Issues of the Dairy Industry: The Survival of Raccoonpox Virus in Milk

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Introduction: America's food supply is at risk of being contaminated by terrorists using pathogens as their agents of biowarfare. The dairy industry is vulnerable to attack because of rapid, widespread distribution of products and easy access to farms, bulk tanks, tanker trucks, and raw milk silos.

Purpose: The objective of this study is to determine the survival of raccoonpox virus (RCN) over a six month period, and to determine RCN survival after pasteurization and high hydrostatic pressure processing. RCN was chosen as a laboratory surrogate to smallpox virus, which is a possible bioterrorist agent.

Methods: Raw milk, skim milk, whole milk, and sterile water samples were inoculated with RCN (10^{-1} dilution) and stored at 4°C in the dark for 6 months. Samples were tested weekly for virus survival using TCID50 with Vero cell culture and titers were calculated. PCR was performed on DNA extracted from milk and cell culture samples. Raw milk samples inoculated with RCN were treated using HTST parameters and high hydrostatic pressure (HHP) to determine RCN survival after these processing treatments.

Results: RCN titer decreased by ~1-log from week 1 to week 24 in skim milk and water samples. In whole milk the titer decreased by ~1.8-log over six months. After one week no cytopathic effect was seen in cell culture from the raw milk samples. PCR results corroborated with the cell culture data. HTST and HHP (≥ 500 MPa) may be effective alternatives to traditional pasteurization for RCN inactivation.

Significance: RCN is a model organism to simulate small pox contamination of food and survives over a six month period in skim milk and whole milk. RCN did not survive past the one week period in raw milk, and it is important to note that RCN survival is far greater than the shelf life of processed milk.

P5-03 Molecular Approaches for the Identification of a Toxin in Foods

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Due to the potential for intentional contamination of food with crude preparations containing ricin, a real-time PCR method was developed for detection of castor plant material in ground beef, milk and liquid eggs. One primer pair was identified and confirmed to be castor-specific and efficient for amplification of the ricin gene in DNA extracts from castor, beef, milk and egg matrices. Of three different DNA extraction protocols compared, the hexadecyl-trimethylammonium bromide (CTAB) method yielded the highest quality of DNA for a real-time PCR assay. The detection limits for castor contamination were < 0.0001% in ground beef, < 0.00001% in milk and liquid egg (corresponding to 0.05 g of ricin/g of beef and 5 ng of ricin/ml of milk or egg). These levels are well below the theshold for human oral toxicity. This real-time PCR method could be used as a fast and accurate alternate detection method for intentional castor contamination of food matrices.

P5-04 Effect of the Combination of pH, Water Activity and Temperature on the Growth of *Bacillus anthracis*

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There is little information about the behavior of *B. anthracis* in foods. In this study we examined the combined effects of pH, water activity and temperature commonly associated with foods on the growth of *B. anthracis*. Brain heart infusion broths of 15 combinations of pH (4.5~8.0) and water activity (0.900~0.995; adjusted with glycerol) were used in the study. There were five tubes (5 ml/tube) for each

combination and each temperature. All tubes were inoculated with stationary phase of *B. anthracis*, Sterne strain, (final population: ca 3 log CFU/ml) and then incubated at 10, 20, 30, 37 or 42°C. Turbidity was used as an indicator of growth (OD600 was measured when applicable). After 4 weeks, there was no visible growth in any a_w/pH combinations incubated at 10°C. Broths with $a_w \leq 0.92$ did not support the growth of *B. anthracis* at any of the temperatures tested. Broths of the following combinations (a_w/pH) showed positive growth of *B. anthracis* during the 4-wk incubation: 0.995/8.0, 0.995/6.1, 0.96/7.1, 0.96/5.2, and 0.94/8.1 at 20°C; 0.995/8.0, 0.995/6.1, 0.96/7.1, 0.94/8.1, and 0.94/6.1 at 30 and 37°C; and 0.995/8.0, 0.995/6.1, and 0.96/7.1 at 42°C. In addition, *B. anthracis* grew in broth with a_w 0.96 and pH 5.3 at 30°C. Our results showed that lower temperature (10°C) or lower a_w (≤ 92) prevented the growth of *B. anthracis* (Sterne). Although *B. anthracis* is usually cultured at 35~37°C, our results indicated it also grew well at 30°C. In fact, it may grow in a broader range of a_w and pH at 30°C than at 37°C. Knowledge of the factors affecting the growth of *B. anthracis* will provide valuable information to evaluate the potential hazard if a food were contaminated with this pathogen.

P5-05 Inactivation of *Bacillus anthracis* Spores by a Combination of Biocides and Heat in Milk at Pasteurization Temperatures

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The milk supply is considered a primary route for a bioterrorism attack with *Bacillus anthracis* spores because typical pasteurization temperatures are not capable of inactivating spores. If so contaminated, an effective way to inactivate the spores in the milk is needed. This study was undertaken to determine the effectiveness of a combined disinfectant/thermal treatment to achieve this end. Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and peroxyacetic acid were evaluated for their efficacy in inactivating strains ANR-1, 7702 and 9131 at 72 to 85°C by use of a sealed capillary tube technique. Strains ANR-1 and 9131 were more resistant to the combined treatment than strain 7702. At 72°C, reductions of more than 6 log CFU/ml of viable spores of strain ANR-1 were achieved by addition of NaOCl at 1.5% (pH 11) in milk within 1.4 min. Similar spore inactivation was achieved at 72°C with 1.5% NaOCl after only 0.7 min if the pH of the biocide was neutral. Treatment of milk with a mixture of 1.5% NaOCl (pH 11) and 0.5% H_2O_2 caused inactivation similar to that with 1.5% NaOCl (pH 7). At 80°C with 1.5% NaOCl (pH 11) and 0.5% H_2O_2, the time to reduce 6 log CFU/ml spores was less than 15 s for all strains. Treatment with a mixture of 1.5% NaOCl (pH 11) and only 0.05% peroxyacetic acid caused 6 log CFU/ml reductions of spores in less than 13 s These results indicate that sodium hypochlorite in combination with hydrogen peroxide or peroxyacetic acid could consistently result in 6-log reductions of *B. anthracis* spores in less than 30 s at temperatures below 85°C. This information will be useful for developing more effective thermal treatment strategies, which could be used in HTST milk plants to process contaminated milk for disposal and decontamination purposes as well as for a potential protective measure.

P5-06 Species-specific Identification of Campylobacters by Use of PCR-RFLP and PCR Targeting the Gyrase B Gene

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Introduction: *Campylobacter* spp. are the most common cause of bacterial gastrointestinal infection in the US and Japan. Species-specific identification of campylobacters is problematic, due to the absence of suitable biochemical assays and the existence of atypical strains. Methods to discriminate and detect *Campylobacter* species are needed to determine their prevalence in food and the environment and their importance in causing human illness.

Purpose: The purpose of this study was to identify sequence polymorphisms in the *Campylobacter gyrB* gene and develop species-specific PCR assays and PCR-RFLP for differentiation of *Campylobacter* species.

Methods: Partial DNA sequences of the *gyrB* gene of 19 strains of *Campylobacter* representing 12 *Campylobacter* species were determined. A universal primer set, designed to amplify a 900-bp fragment of the gyrB gene of each *Campylobacter* spp., was developed and used for PCR-RFLP analysis. Species-specific primer sets targeting the 12 *Campylobacter* species were designed for direct identification of the different species by PCR.

Results: The topology of the resulting phylogenic neighbor-joining tree based on the *gyrB* gene was similar to a previously reported phylogenic tree based on the 16S rDNA gene. However, *gyrB* provides a better resolution for *Campylobacter* species than the 16S rDNA gene. A universal primer set, designed to amplify a 900-bp fragment of the *gyrB* gene in *Campylobacter* spp., was developed and used for PCR-RFLP analysis to differentiate 12 *Campylobacter* species. Digestion of the 900-bp fragment with restriction enzymes resulted in unique digestion patterns for all 12 *Campylobacter* species. Moreover, species-specific primer sets targeting the 12 *Campylobacter* species, yielding PCR products ranging in size from 86 to 482 bp, were specific and useful for identification of each *Campylobacter* species by the PCR.

Significance: PCR-RFLP analysis and PCR using species-specific primer sets based on the *gyrB* gene provide valuable tools for rapid detection and unambiguous identification of the majority of *Campylobacter* species.

P5-07 DSC DNA Microarrays for Genotyping and Population Studies of *Campylobacter jejuni*

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Introduction: *Campylobacter jejuni* is a major cause worldwide of foodborne bacterial gastroenteritis. The continued development of more effective and infor-

mative-typing methods is necessary to improve our understanding of the epidemiology and population dynamics of this important pathogen. Comparative genome indexing (CGI) using whole genome DNA microarrays is a method useful not only for molecular typing, but also to provide considerable strain-specific genetic information unavailable with current typing methods.

Purpose: The purpose of this study was to identify genes with a high degree of strain diversity to indicate host source and US region for use in the development of a novel microarray-based typing method for *C. jejuni*.

Methods: One hundred thirty-three geographically diverse *C. jejuni* isolated from humans, cattle and chickens were typed by CGI. Statistical methods appropriate for data normalization, establishing the presence and absence of genes and highly variable genetic loci, were determined. These methods were used to identify *C. jejuni* genes characteristic of geographic region and host source.

Results: A statistical analysis of the CGI data revealed over 100 genes out of the 1927 genes present on the microarray that varied significantly between the test strains and the type strains (NCTC11168 and RM1221). These genes differentiated isolates by source and the US region from which they were isolated. Of these 100 significantly variable genes, approximately one third can be used to determine the geographic region and the remainder can be used to identify the host source.

Significance: CGI provides a unique tool for genotypic and population studies of *C. jejuni*. The identification of genomic regions with high diversity including source and region specific marker genes forms the basis for development of a novel, more efficient typing method for *C. jejuni*.

P5-08 DSC Genotypic Characterization of Verotoxin-producing *Escherichia coli* Isolated from Ground Beef Samples

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Enterohemorrhagic *Escherichia coli* (EHEC) have emerged as a leading cause of foodborne disease outbreaks in the United States. It is one of the most important foodborne human pathogen of animal origin, with cattle generally considered to be the major reservoir. The objectives of this study were to determine the biochemical profile and the presence of the verotoxin (VT) genes of EHEC isolates from ground beef samples. Fifty ground beef samples were analyzed from area (Huntsville, Alabama) grocery stores for EHEC strains, using modified tryptic soy broth with novobiocin as pre-enrichment broth and cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) as a selective media. The isolates were characterized for their glucoronidase activity, utilization of sugars using API 20 E strips, reaction to O157 and H7 antisera, and the presence of verotoxin (VT 1 and VT 2) and *E. coli* effacement attachment (*Eae*) genes by multiplex PCR assay. Fourteen beef samples (28.0%) were positive for *E. coli*. A total of 150 isolates were obtained, of which 42 (28.0%) were O157:H7, 14 (9.3%) O157: non H7 and 94 (62.7%) non O157:H7. Of the 42 O157:H7 positive isolates, 10 (23.8%) isolates gave positive PCR for Verotoxin (VT) and *E. coli* effacement and attachment (*Eae*) genes. These results indicate that the epidemiology of VTEC strains isolated from ground beef is of major concern and that the use of a multiplex PCR assay applied in combination with serological and enrichment steps is an effective method for isolating verotoxin-producing *E. coli* from ground beef.

P5-09 DSC Gene Expression Analysis of *Escherichia coli* O157:H7 at 10 and 37°C by Use of High Density Oligonucleotide Microarrays

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Introduction: *E. coli* O157:H7 can survive under refrigeration, resulting in enhanced resistance to acidic conditions; research is being pursued to understand its survival mechanisms in foods. Cold shock proteins are induced in *E. coli* O157:H7 when the temperature shifts from 37 to ≤ 20°C, leading to membrane and fatty acid changes and suppression of protein and nucleic acid synthesis. Microarrays can be used to elucidate mechanisms of bacterial survival by determining gene expression.

Purpose: Gene expression analysis of *E. coli* O157:H7 EDL933 cultured at 10 and 37°C was assessed by use of high density oligonucleotide microarrays.

Methods: Cells were grown to 7 log CFU/ml in Luria-Bertani broth (3 reps) at 10°C (refrigeration) and 37°C (control), followed by an optimized total RNA extraction and hybridization onto Affymetrix GeneChip® *E. coli* Genome 2.0 Array, and then confirmation by real-time reverse-transcriptase PCR (rt-RT-PCR).

Results: Microarray results (complete randomized block design; $P < 0.001$) showed 293 down-regulated and 375 up-regulated genes. Cold shock genes, *cspE*, *cspA*, *cspG*, and *cspH* were down-regulated; recA and SOS DNA repair genes, *uvrB*, *yebG*, *ruvA* and *B*, *lexA*, and *dinI* were up-regulated. Expression of *fhuA* (an outer membrane protein) and *napG* (ferredoxin-type protein involved in electron transport) genes was 115- and 70-fold greater under refrigeration; their expression, along with *hemX* (methylase-type gene; an internal control), was confirmed by rt-RT-PCR. *HemX* was detectable up to a 4-log dilution in both control and refrigeration samples; *fhuA* and *napG* were detected up to a 7-log dilution in the refrigerated samples and not detected in the control.

Significance: Results from this study indicate that SOS DNA repair, outer membrane, and electron transport genes were utilized for survival under refrigeration conditions. These data should aid in the design of inactivation methods for *E. coli* O157:H7 in foods.

P5-10 Cloning of Genes Encoding an Antibacterial Peptide Salmine and Construction of Its Expression Vector in *Escherichia coli* BI21

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Introduction: Protamine is a group of simple and highly cationic peptides found in fish sperm. Many protamines, including salmine, have been reported to exhibit broad spectrum of antimicrobial activities and have great potential for application as natural and safe food preservatives for preserving food quality and enhancing food safety.

Purpose: The purposes of this study were to clone and express the genes encoding salmine in *Escherichia coli* BL21 and improve the expression conditions of genetically engineered bacteria strain (*E. coli* BL21-pED-SAL).

Methods: Three primers were designed for cloning genes encoding salmine, using the preferred codons of *E. coli*. The cloned gene fragments obtained from primer complementary PCR were cloned into the pED plasmid a reconstructed by inserting a modified gene fragment encoding the C-terminal peptide fragment of L-asparaginase (L-ansB-C) into the BamHI and HindIII sites of pET28a vector. The recombinant expression vector was expressed in *E. coli* BL21. The expression of the recombinant DNA was analyzed by SDS-PAGE and quantified by Hybridizer System with Acquisition and Analysis Software.

Results: DNA analysis showed that the sequence of the cloned gene contained in the recombinant expression vector was identical to the primers designed for cloning genes encoding salmine. SDS-PAGE analysis showed that the expression of the recombinant DNA in *E. coli* resulted in an expected fusion protein product of 20 KDa. The yield of fusion protein was significantly affected by culture media, time and temperature of fermentation, and inducing time. The highest expression level of fusion protein (40.1%) could be achieved when genetically engineered bacteria strain (*E. coli* BL21-pED-SAL) was fermented in tryptic soy broth at 43°C for 2–4 h, followed by inducing with lactose (5 mM) for additional 8–10 h.

Significance: This study reported the possibility of developing a genetic procedure for producing protamine instead of extracting it from fish sperm.

P5-11 Effect of Salt on the Survival and Cytoplasmic pH of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* in Acetic Acid Treatment

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Organic acids inhibit microorganisms by decreasing the cytoplasmic pH of microorganisms in the acidic environment, and the inhibitory effect could be different when salt is added to acid. Therefore, this study was conducted to investigate the effect of salt on the survival of foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* and on cytoplasmic pH and electrical membrane potential (EMP) of each pathogen with acetic acid treatment. Scanning/transmission electron microscopy (SEM/TEM) was applied to investigate the inhibitory mechanism of acetic acid against *E. coli* O157:H7. Cytoplasmic pH and EMP were estimated by measuring the distribution of a weak acid (radiolabeled salicyclic acid) across the cell membrane. SEM and TEM photographs showed that inhibition of microorganisms by acetic acid occurred because of the decrease of cytoplasmic pH and a loss of cell components. The addition of salt significantly increased the survival of *E. coli* O157:H7 in acetic acid treatment and showed an antagonistic effect, whereas it enhanced the inhibitory effect of acetic acid for killing *L. monocytogenes* and *S. aureus* and thus showed a synergistic effect. These results were in agreement with results of cytoplasmic pH of each pathogen because cytoplasmic pH increased in *E. coli* O157:H7 when salt was combined with acetic acid treatment, whereas cytoplasmic pH decreased in *L. monocytogenes* and *S. aureus*. However, there was no significant difference in levels of EMP between acetic acid and the combination of acetic acid and salt for all strains studied. From these findings, the addition of salt produced different results depending on pathogen strain, and for *E. coli* O157:H7, the addition of salt increased its survival because salt may prevent the acidification of the cytoplasm of *E. coli* O157:H7 by organic acids.

P5-12 Survival Mechanism of *Escherichia coli* O157:H7 in the Combined Treatment of Acetic Acid and Salt Related to the Known *E. coli* Acid Resistance Response

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Escherichia coli O157:H7 is highly resistant to acidic pH and can survive and grow in acidic foods and cause foodborne illness. This resistance of *E. coli* O157:H7 to an acid environment could be related to an acid resistance (AR) mechanism of *E. coli*. In our previous study, the addition of salt significantly increased the survival of *E. coli* O157:H7 with acetic acid treatment and this may happen because the addition of salt increased the AR mechanism of *E. coli* O157:H7. Therefore, this study was conducted to investigate the effect of salt on the AR mechanism of *E. coli* O157:H7 in acetic acid treatment in laboratory media. To investigate the effect of salt addition on *rpoS* regulation (AR1) in acetic acid treatment, a *rpoS* mutant strain and chloramphenicol were used. Glutamate and arginine were added to phosphate buffer to investigate the effect of salt addition on amino acid-dependent systems, AR2 and AR3, in acetic acid treatment. Related to the AR1 system, the same antagonistic effect of acetic acid and salt was observed in *rpoS* mutant strains and in the presence of chloramphenicol. Therefore, the antagonistic effect was not the result of protein synthesis by the AR1 system. However, the addition of glutamate to phosphate buffer significantly increased survival levels of

E. coli O157:H7 in acetic acid treatment. Thus, the protective effect of salt in acetic acid treatment may result because the addition of salt increases the AR response of *E. coli* O157:H7, such as glutamate-dependent AR systems. However, since known AR systems could not sufficiently explain the acid resistance of *E. coli* and the effect of incorporating salt into acid treatment, further study is required to better understand the antagonistic effect of salt and acetic acid.

P5-13 Survival of *Escherichia coli* O157:H7 Isolates in Acetic Acid Solutions is Influenced by the Source of Isolation

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Enterohemorrhagic *Escherichia coli* O157:H7 is highly acid resistant in nature because of its low infectious dose and is able to survive in acid foods or in stomach acid. A number of studies on the influence of acid on *E. coli* O157:H7 have shown considerable strain associated differences, but there is little information on *E. coli* O157:H7 strains isolated from common sources. Thus, the purpose of this study was to determine the survival of five group mixtures of *E. coli* O157:H7 strains (5 mixtures of each: food, bovine carcass, bovine feces, water, and human isolates) in acetic acid solutions (400 mM), with ionic strength adjusted to 0.34 (using NaCl) at different pH values (3.3, 3.7, and 4.3) and temperatures (15, 22, and 30°C) for 25 min exposure. Bovine carcass, feces or water isolates survived acetic acid treatment significantly ($P \leq 0.05$) better than strain mixtures isolated from acidic foods or human isolates at pH 3.3 and 30°C for 25 min exposure. However, resistance to acetic acid significantly increased as temperature decreased to 15°C for a given pH, with little difference ($P \geq 0.05$) among strains. All groups of *E. coli* O157:H7 strains showed more than 4 to 5 log reduction at pH 3.3 and 30°C. Significantly reduced lethality (less than 2 log reduction) for all *E. coli* O157:H7 strain mixtures was observed as pH increased to 3.7 or 4.3, with little difference in acetic acid resistance among groups. In the meantime, the addition of glutamate in the acetic acid solution or anaerobic incubation provided the best protection compared to the above condition for all groups of isolates. These results may suggest strategies for improving the safety of acidified foods.

P5-14 DSC Starvation Effect on Attachment Properties of *Escherichia coli* O157:H7 and a Non-pathogenic Surrogate *E. coli*

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Introduction: Water is widely used in fresh produce production. *E. coli* O157: H7 can survive in water for extended periods, which induces starvation. Exposure to starvation may enhance *E. coli* O157:H7 survival under subsequent stresses by changing surface morphology, which may affect adherence to produce.

Purpose: This study determined the effect of starvation on attachment of *E. coli* O157:H7 and a surrogate *E. coli* ATCC 25922 to lettuce.

Methods: After starvation (at 37°C for 4 h, 20°C for 24 h, or 4°C for 7 d), cryotolerance, cell surface characteristics (hydrophobicity, zeta potential, morphology and outer membrane proteins) and attachment to lettuce were investigated.

Results: Overall *E. coli* ATCC 25922 exhibited greater starvation induced changes and cryotolerance than *E. coli* O157:H7. For example, starved (4°C for 7 d) *E. coli* ATCC 25922 showed a 17.6% increase in survival rate, whereas the corresponding increase for *E. coli* O157:H7 was 8.8%. Hydrophobicity of both strains decreased over 7 days of storage and zeta potential of *E. coli* ATCC 25922 was more consistent (-8.08 to -6.98 mV) than for *E. coli* O157:H7 (-10.30 to -6.21 mV). There were few differences in outer membrane protein (OMPs) patterns among starvation conditions, whereas intensity of some OMPs of *E. coli* ATCC 25922 was different from intensity of *E. coli* O157:H7. Microscopically starved cells of both strains appeared elongated, wrinkled and malformed. Starved cells of both strains attached to lettuce less than non-starved cells (49% of *E. coli* O157:H7 and 69% of *E. coli* ATCC 25922 compared to non-starved cells).

Significance: Starvation affects cryotolerance, cell surface properties of *E. coli* O157:H7 and *E. coli* ATCC 25922 to somewhat different degrees. Thus selection of a surrogate should be done under specific environmental conditions. *E. coli* ATCC 25922 is a useful surrogate for *E. coli* O157:H7 for produce attachment and recoverability studies.

P5-15 The Lpp Lipoprotein Suppresses Motility in a Biofilm-forming Strain of *Escherichia coli* O157:H7

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Introduction: In *Escherichia coli* O157:H7 strain ATCC 43895, a guanine to thymine transversion in the *csgD* promoter created strain 43895OR. Strain 43895OR produces an abundant extracellular matrix rich in curli fibers and generates dense biofilms on solid surfaces.

Purpose: Investigate the components of the extracellular matrix of strain 43895OR and their role in *E. coli* persistence in food environments.

Methods: The soluble proteins from strains 43895OR and 43895 grown on Congo red indicator (CRI) plates were compared by one-dimensional PAGE. Two unique proteins from strain 43895OR were identified by immuno-blotting and mass spectrometry. Mutants of strain 43895OR with disruption of each identified gene were created and tested for phenotypic and functional differences on CRI agar and on motility plates (0.3% agar). Transcriptional expression of the identified genes in strains 43895 and 43895OR was determined by quantitative real-time reverse-transcriptase PCR.

Results: A CsgA polyclonal antibody identified a 15-kDa protein as the curli subunit. A <10-kDa protein was identified from MALDI-TOF combined MS+MS/MS spectra as a murein-lipoprotein Lpp homologue. Comparison of strains 43895 and 43895OR with *lpp*, *csgA*, or *lpp/csgA* mutants revealed differences in aggregation on CRI plates and in broth. In motility assays, the *lpp* mutant and the *csgA/lpp* double mutant each showed significantly ($P < 0.05$) greater swimming motility than that strain of 43895OR, strain 43895, or the *csgA* mutant. However, there were no motility differences between the lpp and the *csgA/lpp* mutant strains. Although *csgA* RNA transcripts were up-regulated in strain 43895OR, *lpp* expression was indistinguishable between strains 43895 and 43895OR.

Significance: These results indicate that curli fibers and Lpp are highly expressed during 43895OR colony growth on agar and that Lpp has a suppressive affect on swimming motility, which could affect survival in a food environment. Moreover, the increased expression of Lpp is not controlled at the transcriptional level.

P5-16 Quorum-sensing Signal Production and Its Role on Growth of *Salmonella* Thompson and *Escherichia coli* O157:H7 under Various Conditions

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Foodborne pathogens use cell-to-cell signaling (quorum sensing) based on autoinducer-2 (AI-2) activity. The potential role of AI-2-based quorum sensing in bacterial growth and the potential production of AI-2 in liquid substrates are not well known. This study evaluated the role of AI-2 on growth of *Salmonella* Thompson and *Escherichia coli* O157:H7, the amount of AI-2 produced by the pathogens in the presence of glucose (0.5%), and production of AI-2 by *E. coli* O157:H7 in beef purge. *S.* Thompson RM1987N (AI-2-positive) and RM1987NLUX (AI-2-negative), and *E. coli* O157:H7 86-24 (AI-2-positive) and VS94 (AI-2-negative) were incubated in Luria-Bertani broth (LB) at 35°C (14 h), and cell counts [tryptic soy agar (TSA)], cell density (OD600), and AI-2 activity were determined every 2 h. *S.* Thompson RM1987N (8 h) and *E. coli* O157:H7 86-24 (14 h) were incubated in LB+0.5% glucose broth (LBG) at 35°C, and analyzed for OD_{600} and AI-2 activity. In addition, *E. coli* O157:H7 ATCC 43895 was inoculated in beef purge, and total bacterial (TSA) and *E. coli* O157:H7 (sorbitol MacConkey agar supplemented with cefixime and potassium tellurite) populations were determined every 7 (4°C), 6 (10°C) and 3 (25°C) days for 21, 18 and 9 days, respectively. AI-2 activity was determined by the luminescence-based reporter *Vibrio harveyi* BB170 (two replications, two samples each). No differences ($P \geq 0.05$) in bacterial populations and cell densities were observed between AI-2-positive and -negative strains. Unlike transient AI-2 activity in LB, the activity became constant in LBG during stationary phase. No ($P < 0.05$) AI-2-like activity was detected in beef purge, even in the presence of high *E. coli* O157:H7 populations. The findings indicate that AI-2-based quorum sensing may not be involved in growth of *S.* Thompson and *E. coli* O157:H7, while an increased glucose level may allow continuous AI-2 activity. AI-2-based quorum sensing of *E. coli* O157:H7 did not appear to be of significance in beef purge.

P5-17 Heat Inactivation Comparison of *Escherichia coli* O157:H7 When Grown Statically or Continuously in a Chemostat

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Introduction: Studies have reported wide ranges of D-values for thermal inactivation of various strains of *Escherichia coli* O157:H7. The focus for much of the research to date has been to model the growth medium and possible stress conditions that lead to variability in thermal resistance. The use of non-linear regression techniques to better model inactivation data that is curvilinear or show tailing effects at later time points in survivor curves has been demonstrated.

Purpose: The objective of this study was to determine if the variability of D-values and possible curvilinear shapes of survivor curves is the result of the physiological state of microbial cells at the time of heat exposure.

Methods: *E. coli* O157:H7 (ATCC strain 43895) was grown in a continuous growth chemostat or aerobically incubated static culture. Culture medium was tryptic soy broth. The chemostat enabled a quasi-constant growth rate allowing bacteria to remain in a sustained or synchronous growth phase. Survivor curves were prepared by heating cultures in vials at 58°C for up to 36 min and recovering survivors on tryptic soy agar. A regression package capable of both linear and non-linear approaches was used to analyze results.

Results: Cultures were ca pH 7.9 (7.5 during heating) for chemostat cells and pH 6.1 (6.4 during heating) for statically grown cells. Regression analysis performed on inactivation curves showed slight differences in R^2 values for log linear versus the non-linear Weibull function, 0.92 to 0.98 respectively for static culture and 0.89 to 0.94 respectively for chemostat culture. Statically grown cells had a significantly larger D58C value of 5.49 min compared to 2.34 min for chemostat cells.

Significance: Results suggest there are differences in the inactivation response of microbial cells depending on their physiological state. Growth conditions and physiological state should be taken into account when modeling validation experiments to ensure adequate food safety.

P5-18 Pathogenicity of Acid-adapted *Escherichia coli* O157:H7 in Laboratory Media and Meat Serum

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Toxicity of acid adapted and non-adapted *Escherichia coli* O157:H7 grown in laboratory media and meat serum on African green monkey kidney cells

(Vero cells) was determined. *E. coli* O157:H7 was adapted to acidic conditions by growing in tryptic soy broth (TSB) with 1% glucose (TSB+ 1%G). A five-strain cocktail of *E. coli* O157:H7 was grown separately in TSB and TSB+1%G for 24 h at 37°C to provide cells with or without acid adaptation. Meat serum was prepared from irradiated ground beef and inoculated with a five strain cocktail of either acid adapted or non-adapted *E. coli* O157:H7. Vero cells were grown separately in complete RPMI media and toxic effects of acid adapted and non-adapted *E. coli* O157:H7 grown in laboratory media and meat serum were compared after storing for 1 and 7 days at 4 and -20°C. No significant ($P > 0.05$) differences were observed between the toxicity of acid adapted and non-adapted cells in laboratory media and meat serum irrespective of storage temperature. Significant differences ($P < 0.05$) were observed in the verotoxin production on day 1 and 7 at 4 and -20°C. Significantly higher ($P < 0.05$) verotoxin production was observed in meat serum inoculated with non-adapted cells on day 1 as compared to acid adapted cells but this was not observed on day 7. These results suggest that acid adaptation of *E. coli* O157:H7 leads to decreased verotoxin secretion in meat serum.

P5-19 Heat Resistance of Multidrug- and Nonmultidrug-resistant *Salmonella* Serotypes

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Introduction: The rate of detecting multidrug-resistant (MDR) strains of *Salmonella* has increased considerably in recent years. It has been hypothesized that MDR strains are generated by sub-therapeutic antibiotic use in food animals and that such strains may have increased resistance to food processes such as heat and irradiation.

Purpose: The objective of this study was to evaluate the heat resistance of MDR and nonmultidrug-resistant (NMDR) strains of ten *Salmonella* serotypes representing the serotypes isolated from ground beef national baseline samples for 2005.

Methods: MDR and NMDR *Salmonella* serotypes studied included: Montevideo, Typhimurium, Anatum, Muenster, Newport, Mbandaka, Dublin, Reading, Agona, and Give. Stationary-phase cultures of the strains were aliquoted into sterile capillary tubes and immersed in a temperature-controlled water bath at 55, 60, 65, and 70°C for appropriate times. Survivor curves were plotted for each temperature and a best-fit linear regression was derived for each temperature. D- and z-values were calculated for each strain.

Results: For MDR strains, the D-values at 55°C ranged from 156–251 s; at 60°C ranged from 9–23 s; at 65°C ranged from 4–5 s; and at 70°C ranged from 1–3 s. For NMDR strains, the D-values at 55°C ranged from 148–265 s; at 60°C ranged from 8–25 s; at 65°C ranged from 3–5 s; and at 70°C ranged from 1–2 s. The z-values for the MDR ranged from 2.7–3.4°C, while the z-values for the NMDR ranged from 2.1–3.3°C. Although there was no substantial difference in the heat resistance of MDR and NMDR strains, MDR strains generally appeared to have slightly lower heat resistance compared to that of NMDR strains. NMDR *S.* Agona consistently had the highest heat resistance of all serotypes evaluated.

Significance: These data provide evidence that *Salmonella* strains with MDR genes do not appear to confer increased resistance to heat compared with NMDR counterparts.

P5-20 The Role of Meat Microflora in Gas Production 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. The authors previously identified the predominant microflora associated with "blown-pack" spoilage of ground beef chubs stored under refrigerated conditions.

Purpose: The objective of this study was to evaluate the role of three bacterial groups (*Enterobacteriaceae*, *Pseudomonas*, and lactic acid bacteria [LAB]) in gas production when inoculated in vitro in a simulated ground beef matrix.

Methods: A full-factorial design was used to evaluate the effect of the three bacterial groups inoculated at three concentrations (2, 3, or 4 log CFU/g), in tubes containing gas-assay media equipped with Durham tubes and incubated at three temperatures (4, 10, or 15°C). The tubes were incubated until visual signs of gas production were observed. A second-order logistic regression model was fitted to the data for each temperature to predict the probability of gas production as a function of level of inoculated organisms.

Results: *Pseudomonas* were found to have no significant ($P > 0.05$) effect on gas production. At 4°C, LAB and *Enterobacteriaceae* were found to have a significant ($P < 0.05$) effect on gas production. At 10 and 15°C, *Enterobacteriaceae* were found to have a significant ($P < 0.05$) effect on gas production. The fitted response surface also predicted levels of organisms for which the probability of gas production is maximum. At 4°C and a combination of 2.9 and 3.0 log CFU/g of *Enterobacteriaceae* and LAB, respectively, the probability of gas production was maximum (68%). At 10 and 15°C with a concentration of 3.0 log CFU/g *Enterobacteriaceae*, the probability of gas production was 71%.

Significance: Use of the developed model will allow meat processors to predict the likelihood of ground beef packages experiencing gas production based on microbiological profiles for the specific organisms of interest.

P5-21 Identification and Molecular Characterization of Class 1 Integron Resistance Gene Cassettes among *Salmonella* Strains from Imported Seafood

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Introduction: In the United States, there are an estimated 800,000 to four million *Salmonella* infections annually, of which approximately 500 of the cases are fatal. Recently, numerous outbreaks have been caused by the multidrug resistant *Salmonella* strains. Mobile genetic elements, such as plasmids, transposon, and integrons, which are able to disseminate antibiotic resistance genes by horizontal or vertical transfer, play an important role in evolution and dissemination of multidrug resistance in Gram-negative bacteria.

Purpose: The purpose of this study was to identify and characterize the multidrug resistant genes among *Salmonella* strains isolated from imported seafoods.

Methods: A total of 202 *Salmonella enterica* isolates representing 20 serovars were isolated from imported seafood samples during the years 2001–2005. These isolates were tested for their susceptibility to thirteen antibiotics of veterinary and clinical importance. Multidrug resistant isolates were screened for integrons by PCR and further characterized by cloning and nucleotide sequencing.

Results: Fifty-five strains were resistant to at least one antibiotic tested. Four strains, Newport (62), Typhimurium varcopen (629), Weltevreden (631) and Lansing (803), which originated in different countries (Hong Kong, Philippines, India and Taiwan), were resistant to multiple antibiotics, including trimethoprim sulfamethoxazole and tetracycline. Primers specific to integron conserved sequence (CS) regions amplified a 1.24 Kb amplicon from strain 62, and a 2 Kb amplicon from the three isolates 629, 631 and 803. Sequence analysis of the 1.24 kb clone of 62 revealed the presence of *dfrA1* conferring resistance to trimethoprim and *orfC* gene cassette of unknown function. The sequence analysis of the 2 kb clone of 629, 631 and 803 revealed the presence of *dhfrXII* gene cassette, which confers resistance to trimethoprim; a 59 base element, ORF of a hypothetical protein; and *aadA2* gene cassette, which confers resistance to streptomycin and spectinomycin.

Significance: This study indicates antimicrobial-resistant *Salmonella enterica* serovars are present in imported seafoods.

P5-22 Characterization of *Salmonella* Isolates from Sentinel Bivalves (*Corbicula flumenia*) Using Serotyping, Pulsed Field Gel Electrophoresis (PFGE), Multi Locus Sequence Typing (MLST) and Antimicrobial Resistance Analysis (ARA)

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Introduction: Among the 21 million acres of shellfish harvesting areas in the United States, more than 13% are closed due to fecal waste contamination from humans, livestock and wildlife.

Purpose: The purpose of this study was to characterize *Salmonella* isolates from sentinel *Corbicula flumenia*, using phenotyping and genotyping in an attempt to identify the origins of fecal waste contamination.

Methods: *Corbicula flumenia* specimens were placed at 15 river locations in North Carolina near human waste water treatment plants and intensive swine rearing areas. For each location, the prevalence of *Salmonella* was determined every two months, in addition to after two storm events, occurring between April 2004 and August 2005. A total of 52 *Salmonella* isolates were obtained and characterized by serotyping, PFGE, ARA and MLST based on one virulence gene and three housekeeping genes. Isolates were compared with those described in the human CDC PulseNet database and a database of isolates from swine.

Results: Of the 52 *Salmonella* isolates characterized, fifteen *Salmonella* serovars were identified. The top five serovars were *Salmonella* Newport (15%), Give (13%), Muenchen (13%), Rubislaw (13%) and Braenderup (12%). Sixteen of these 52 *Salmonella* isolates (31%) were identical to human isolates by PFGE and serotyping. Another 9 isolates (17%) had greater than 90% similarity to human isolates. The strains of several PFGE types were further differentiated by MLST. Forty-six percent of the *Salmonella* isolates were resistant to at least one antibiotic. A high percentage of the *Salmonella* isolates was resistant to streptomycin and sulfasoxasole.

Significance: The combination of serotyping, PFGE, MLST and ARA provide opportunities for examining the relationship between environmental isolates and those that have been associated with outbreaks of salmonellosis. Sentinel bivalves can provide a useful cumulative profile of enteric pathogens that may be entering surface waters.

P5-23 DSC Multiplex PCR for Identification of Major Pathogenic *Salmonella* Serovars Obtained from Specific Primer Sets by Use of Comparative Genomics

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Introduction: Salmonellae are divided taxonomically into two species, *Salmonella enterica* and *Salmonella bongori* (subspecies V). *Salmonella enterica* is comprised of 6 subspecies such as I, II, IIIa, IIIb, IV, and VI. *Salmonella* subspecies I consist of almost 1500 serovars, and most major pathogenic *Salmonella* serovars belonging to subspecies I cause infection in warm-blooded animals. *Salmonella* spp. is the most frequently isolated foodborne pathogen from foodborne outbreaks throughout the world and a method for its detection is necessary in the food industry.

Purpose: The purpose of this study is to design specific primers for *Salmonella*, *Salmonella* subspecies I, *Salmonella enterica* serovar Typhimurium, and *Salmonella* Typhi, using comparative genomics, and to identify salmonellae by multiplex PCR, using these primers.

Methods: Specific primer sets were designed from 12 *Salmonella* genome sequences, using comparative genomics, and each primer was evaluated with genomic DNAs of various *Salmonella* serovars. Also, a set of multiplex PCR primers was designed and performed for rapid identification of salmonellae and major pathogenic *Salmonella* serovars.

Results: Specific expected genes were selected from genome sequences of salmonellae by use of comparative genomics, and primers were constructed. Each primer pair showed high specificity with their target serovars, and specific primer pairs were selected for *Salmonella*, *Salmonella* subspecies I, *Salmonella* serovar Typhimurium and Typhi. Also, multiplex PCR results showed specific results with genomic DNAs of various *Salmonella* serovars.

Significance: This multiplex PCR method could apply to the detection and identification of clinically important strains of *Salmonella* serovars with rapidity and accuracy in the general laboratory.

P5-24 DNA Probe Enumeration and rep-PCR Molecular Typing for Monitoring *Salmonella enterica* from Environmental Sources

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Introduction: Salmonellosis often results from fecal contamination of food or water, but *Salmonella enterica* may become established in aquatic environments and food products. Thus, species-specific monitoring is needed for epidemiological analysis.

Methods: Investigations examined an alkaline phosphatase-labeled oligonucleotide DNA probe, derived from the *invA* gene, as a confirmatory tool for identification and enumeration of *S. enterica* from environmental samples. Probe positive colonies were isolated from plate counts or from most probable number (MPN) enrichment protocol of Suwannee River water samples. Serology and repetitive extragenic palindromic DNA (rep-PCR) were used for species confirmation.

Results: The probe proved 100% specific for 31 different serotypes of *S. enterica* strains (n = 83) and negative for all non-*S. enterica* strains (n = 44) of different species (n = 16). The probe detected 10 CFU ml^{-1} in artificially inoculated oyster homogenates or Suwannee River water. Counts using colony hybridization averaged >80% at inocula for 10^2 to 10^6 CFU ml^{-1}, and were significantly ($P < 0.005$) improved counts using selective media. *S. enterica* was isolated from Suwannee River water by combining a most probable number (MPN) enrichment protocol with DNA probe confirmation. All probe positive isolates (n = 326) were confirmed by two independent PCR assays; however, examination of a subset of strains (n = 30) showed that 20% were not typeable by serology. Conversely, all strains were identified as *S. enterica* subspecies I by PCR-based molecular typing using primers from repetitive extragenic palindromic DNA (rep-PCR). Furthermore, rep-PCR provided increased discrimination compared to PCR-based ribotyping, segregating Suwannee River strains from those of clinical origin, and showing excellent correlation with serotyping.

Significance: DNA probe offers rapid identification and enumeration of *S. enterica*, and rep-PCR was more accurate and sensitive than serology for classification of environmental reservoirs.

P5-25 DSC Characterization of Plasmids Harboring the *bla*CMY-2 Gene Encoding Resistance to Extended-spectrum Cephalosporins in *Salmonella* Isolated from Food-producing Animals in Canada

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Introduction: *Salmonella* from animals can be transmitted to humans along the food chain, and antimicrobial resistance in *Salmonella* of animal origin has become a great concern for food supply and public health. This is particularly true for the recently emerged resistance to extended-spectrum cephalosporins (ESCs). Molecular epidemiology is a valuable tool to help us understand the ecology of antimicrobial resistance in animals and to take appropriate measures for its control.

Objective: Plasmids carrying the *bla*CMY-2 gene that confers resistance to the ESCs in *Salmonella* spp. were characterized. The isolates were collected from animals, their environment, and foods of animal origin across Canada during the years of 1999–2004.

Methods: We examined *bla*CMY-2 plasmids from 46 representative isolates selected among a collection of 652 ESC-resistant *Salmonella*. The plasmids were electroporated into *Escherichia coli* DH10B, and purified, after which restriction fragment length polymorphism (RFLP) analysis was performed, using the endonuclease *BglII*. The similarities between restriction profiles of *bla*CMY-2 plasmids were assessed by use of the Dice coefficient and the UPGMA method of clustering.

Results: A variety of *bla*CMY-2 plasmids ranging in size and RFLP pattern were observed. Three major *bla*CMY-2 plasmid clusters were delineated. Plasmids from two of these clusters encoded resistance to several antimicrobial classes, whereas those from the third cluster encoded resistance to ß-lactams only. Plasmids from more than one cluster were found in some *Salmonella* serovars, as well as among *Salmonella* associated with each animal species, except for cattle.

Significance: These results show that a diversity of plasmids carry the *bla*CMY-2 gene in *Salmonella* associated with food-producing animals in Canada. They suggest the repeated acquisition and frequent horizontal transfer of *bla*CMY-2 plasmids in *Salmonella*. Finally, some differences may exist between animal species with regard to the distribution of these plasmids.

P5-26 Alteration of Flagella and Lipopolysaccaride on Biofilm Formation by *Salmonella enterica* Serovar Typhimurium Phage Type DT104

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The increase of multidrug-resistant *Salmonella* has been a public health concern. In a previous study,

multidrug-resistant *Salmonella enterica* serovar Typhimurium phage type DT104 was shown to be able to form strong biofilms on food contact surfaces. Biofilm formation by the pathogen could be a source of cross contamination during handling and processing of food products. Therefore, the objective of this study was to characterize the molecular mechanisms of biofilm formation by S. Typhimuriun DT104 for a better understanding of its attachment and colonization in food processing environments. Random genomic DNA mutagenesis was carried out using the EZ:TN <R6yori/Kan-2> Tnp transposome kit. The clones harboring the EZ:TN transposon were screened by phenotypic analysis of their biofilm and pellicle formation, curli synthesis and cellulose expression. The sites of transposon insertion in selected mutants were sequenced, and these mutants were tested for their biofilm-forming capability in meat, poultry, and vegetable broths or on food contact surfaces. The effect of phenotypic changes of flagella and lipopolysaccaride (LPS) production on biofilm formation by the mutants was determined by SDS-PAGE analyses. Mutants in the *flgK* gene encoding flagellar hook-associated protein 1 and *rfbA* gene encoding glucose-1-phosphate thymidylyltransferase showed decreased biofilm formation in beef, pork, chicken, and turkey broths. These mutants also showed phenotype changes in pellicle formation and curli expression. Alteration of flagella and LPS production were confirmed in the *flgK* mutant and *rfbA* mutant, respectively. The attachment of two mutants on surfaces of stainless steel and glass was insignificant compared to that of wild-type strain ($P < 0.05$). Our findings suggest that *flgK* and *rfbA* genes involved in the biosynthesis of flagella or LPS could contribute to biofilm formation and attachment of *S.* Typhimuriun DT104 on food contact surfaces and raw materials of meat and poultry products.

P5-27 DSC Comparison of Invasiveness and Virulence Potential of Processing Plant Persistent and Clinical Strains of *Listeria monocytogenes* in Different Virulence Models

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Listeria monocytogenes is a serious foodborne bacterial pathogen prevalent in fishery products. Recently, one group of genetically similar *L. monocytogenes* strains (RAPD type 9) have been shown to be persistent in several independent fish industries. As persistent strains are likely to become contaminants of food products, it is important to know how virulent such strains are. The aim of this study was to investigate the virulence of six different *L. monocytogenes* strains in four virulence models; an epithelial cell line, Caco-2; a nematode model, *Caenorhabditis elegans;* a fruit fly model, *Drosophila melanogaster;* and a guinea pig model. The six strains included the EGD strain, the Scott A strain, one human clinical strain, one strain isolated from food and two persistent strains (RAPD type 9) from fish processing environments. The two persistent strains did not invade Caco-2 cells (10^2 CFU/ml) as well as the four other strains (10^4–10^5 CFU/ml) ($P < 0.05$). The persistent strains killed 50% of the nematodes more slowly (110 h) than the rest of the strains (80 h). In the *D. melanogaster* model, the non-virulent *Listeria innocua* behaved similarly to Scott A, and the model is therefore not an appropriate model for *Listeria* spp. Scott A and one persistent strain were tested in the guinea pig model. Scott A was isolated from feces at a higher level (approx. 10^5 CFU/g) than the persistent strain (approx. 10^2 CFU/g) ($P < 0.05$). Further, Scott A was detected in 70-100% (n = 18) of the organs (liver, spleen, jejunum), whereas only 0–40% (n = 27) of the organs were positive when the animals were dosed with the persistent strain. All together, the results suggest that the two processing plant persistent process strains (RAPD type 9) were less invasive and less virulent than Scott A and the human clinical strain.

P5-28 DSC Analysis of the Partial Genomic Sequence among Listeriaphage 20422-1, 805405-1 (Newly Isolated in the United States) and P100

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Introduction: *Listeria monocytogenes* is responsible for human listeriosis, a foodborne illness with high mortality. Listeriaphage has been studied to control *L. monocytogenes* in food and in the processing plant environment. In this investigation, two Listeriaphages (20422-1 and 805405-1) were isolated from environmental samples of two different turkey processing plants located in different states in the United States.

Purpose: This study was performed to determine the similarity of the partial genomic sequence among 20422-1, 805405-1, and P100.

Methods: Eight randomly chosen regions in P100 (genomic sequence was provided by NCBI BLAST) were used to design primer sets, and, after PCR amplification of those regions in the 20422-1 and 805405-1 genome, the amplicons were sequenced.

Results: Listeriaphage 20422-1 and 805405-1 showed exactly the same sequence (100% identity) even thought the phages were isolated in two different states. In the sequenced regions these phages also showed 93–100% indentity in nucleic acid sequence with P100, which was isolated from a sewage sample of a dairy processing plant in Germany in the late 1990s.

Significance: The findings suggest a common pool of virulent phage that can infect *Listeria* in different plants and different nations.

P5-29 DSC Role of Growth Temperature on Freeze-thaw Resistance of *Listeria monocytogenes*

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Listeria monocytogenes is a gram-positive, facultative anaerobic foodborne pathogen that has the ability

to grow at refrigeration temperatures. Currently this organism accounts for a major fraction of deaths due to foodborne illness in the United States. Most outbreaks of listeriosis are associated with bacteria of serotype 4b. In many microbial systems, cold shock and cold acclimation result in enhanced tolerance of the organisms to freezing and thawing. However, few studies have focused on cryotolerance in *Listeria*. In this study, we investigated the impact of growth of *L. monocytogenes* serotype 4b at refrigeration temperatures and at 37°C on tolerance to freeze-thaw stress. Strains implicated in major outbreaks, as well as strains obtained from the food processing plant environment, were characterized. Following growth at 4°C or 37°C, cells were frozen at -20°C, and repeated freeze-thaw cycles were applied every 24 h. After 18 cycles for 4°C-grown *L. monocytogenes* strains 1403 and 1450 (epidemic clone ECI and ECII, respectively) population reductions were 4.34 + 0.34, and 2.77 + 0.07 log CFU/ml, respectively. On the other hand, when these strains were grown at 37°C the log decreases after 18 cycles were only 0.71 + 0.17 and 0.23 + 0.07, respectively. Other strains of serotype 4b and the selected strains of other serotypes also showed negative impact of growth at low temperature on freeze-thaw tolerance. In addition, we found that freeze-thaw tolerance of 37°C -grown cells was significantly higher in stationary phase bacteria than in bacteria at logarithmic phases of growth. Although increased tolerance to freeze–thaw and other stresses in stationary phase cultures has been observed in several other systems, the negative impact of growth at low temperature was unexpected, and has not been reported before with this or other psychrotrophic microorganisms.

P5-30 Membrane Fatty Acid Modifications of *Listeria monocytogenes* Scott A Biofilms Grown at Room Temperature and 4°C on Stainless Steel and Polyoxymethylene (Delrin®)

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Planktonic cells of *Listeria monocytogenes* have been shown to respond to changing growth conditions by varying membrane fatty acids that contribute to membrane fluidity and allow survival at refrigeration temperatures. As bacterial cells alter the fatty acid composition of their lipids, factors such as hydrophobicity and adhesion ability can also be affected. This research examined the variations in fatty acids present in the cell membrane of *L. monocytogenes* Scott A grown in a CDC biofilm reactor on materials commonly used in food processing, namely stainless steel and the acetal resin, polyoxymethylene (PMO) (Delrin®) at room temperature and 4°C. After sufficient growth, cells were harvested and fatty acids were extracted and analyzed by gas chromatography. Statistical analysis of fatty acid profiles displayed differences related to both temperature and the biofilm attachment surface type. Greatest variation was seen on PMO at 4°C, where fatty acids 11:0, 2-OH-12:0, i16:0 and 2OH-16:0 were all present at higher concentrations as compared to growth on stainless steel. Growth at room temperature on PMO resulted in more anteiso-15:0 and iso-15:0 fatty acids present in the cell membrane as compared to stainless steel; however, at 4°C, there was no difference in these two fatty acids on PMO and stainless steel. Growth on stainless steel at room temperature resulted in a profile with more 16:0 than growth at 4°C; however, this fatty acid remained constant at these two temperatures when grown on PMO. Growth on PMO and stainless steel resulted in higher levels of cis-18:19 except on PMO at 4°C. Previous research has indicated that the use of hydrophobic surfaces in cold rooms may minimize the development of *L. monocytogenes* biofilms in food plants. The changes in fatty acid profiles observed in this study may be related to the organism's surface adhesion ability and hence its survivability.

P5-31 Survival of Surface Attached *Listeria monocytogenes* in Drying Up Stress Models

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The presence of *Listeria monocytogenes* in Ready-to-eat food is caused primarily by contamination by persistent strains during processing. We have found that one group of genetically similar *L. monocytogenes* strains frequently dominates and persists in several fish slaughter and smokehouses. One of the fish slaughterhouses harboring this group of *L. monocytogenes* strains manufactures only during the months of October to December. The processing plant is then left dry during the remaining nine months after a "spring cleaning." In September 2006, 46 swab and sponge samples were collected from the processing equipment after nine months without manufacturing. Six samples tested positive for *L. monocytogenes*. The RAPD types for all positive samples were identical to the persistent in-house *L. monocytogenes* RAPD type that had persisted in the slaughterhouse since 2002 and found in each manufacturing season. The fact that these strains survived in the dry processing plant during the non-manufacturing season may indicate they have a remarkable ability to survive under dry conditions. In light of this finding, the survival of different strains of *L. monocytogenes* of different origin (processing equipment, human, culture collection, and environment) during dehydration conditions was compared. Strains of *L. monocytogenes* were allowed to attach to polystyrene microtiter plates or to stainless steel coupons, and all survived for months at 15°C at low humidity in an excicator. Cell numbers were reduced to approx. 0.1% during the first 24 h of the drying up process (Lm recovered/Lm inoculated x 100). No differences or minor survival were found between strains, and *L. monocytogenes* strains appear to have a common ability to survive drying up when attached to surfaces. This may partly explain their remarkable ability to persist in processing environments.

P5-32 Survival of Thirteen *Listeria monocytogenes* Strains in a Dynamic Model of the Stomach and Small Intestine

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Introduction: Being able to survive passage though the human gastrointestinal tract may be linked to the ability of *Listeria monocytogenes* to establish infection.

Purpose: We examined differences in survival among thirteen *L. monocytogenes* strains (including 10403S and its *sigB* mutant, A1-254), representing different serotypes (1/2, 1/2a, 4a, 4b) and three genotypic lineages, using a dynamic gastrointestinal model.

Methods: *L. monocytogenes* counts were determined (in triplicate; PALCAM, tryptic soy agar plus 0.6% yeast extract) during the gastrointestinal challenge (gastric, for 120 min; intestinal, for 240 min; 37°C) following mixing of 4 or 16-h cultures (tryptic soy broth without dextrose plus 0.6% yeast extract) with artificial saliva. Parameters included gastric emptying and gastrointestinal fluid secretion rates, gradual gastric acidification (pH reduction to 2.00 within 88 min), and intestinal pH maintenance (6.50 ± 0.30). Inactivation curves in each gastrointestinal compartment were fitted by use of the Baranyi model.

Results: Initial *L. monocytogenes* counts were 7.0–8.0 log CFU/ml (4-h cultures) or 8.0–9.0 log CFU/ml (16-h cultures). Strain gastric inactivation rates (log CFU/ml/min) ranged from -0.262 ± 0.022 to -0.002 ± 0.001 and from -0.221 ± 0.013 to -0.009 ± 0.001, for 4 and 16-h cultures, respectively. Trends in gastric survival were similar between 4 and 16-h cultures, except for strains N1-227 (4b, lineage 1), A1-254 (1/2a, lineage 2), and J1-158 (4b, lineage 3), which exhibited greater ($P < 0.05$) acid-sensitivity as 16-h cultures. Differences in gastric survival among strains were observed mainly after 120 min of exposure. Counts reaching the intestine 30 min after digestion were 5.4 ± 0.4 to 8.1 ± 0.3 log CFU/ml (4-hour cultures) and 7.0 ± 0.5 to 9.1 ± 0.1 log CFU/ml (16-h cultures). Intestinal inactivation rates (log CFU/ml/min) were -0.016 ± 0.004 to -0.004 ± 0.005 (4-h cultures) and -0.021 ± 0.013 to 0.000 ± 0.003 (16-h cultures). Although strains C1-056 and Scott A displayed the highest acid-sensitivity, levels recovered from the intestine were > 5 log CFU/ml, even after the 240-min challenge. No serotype or lineage-related trends were identified ($P \geq 0.05$) in this study.

Significance: Results suggest that *L. monocytogenes* levels on a food may affect cell numbers in the gastrointestinal tract more than differences in acid resistance among strains.

P5-33 Inactivation of Barotolerant Strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 by High-pressure and Tert-Butylhydroquinone Combination

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Introduction: Antimicrobial efficacy of ultra-high-pressure (UHP) can be enhanced by application of additional hurdles. Previously, tert-butylhydroquinone (TBHQ) was found to sensitize *Listeria monocytogenes* to UHP. To test the feasibility of using this combination in food, synergy between UHP and TBHQ should be demonstrated against barotolerant Gram-positive and Gram-negative pathogens.

Purpose: The objective of this study was to systematically assess the enhancement in pressure lethality by TBHQ treatment, against barotolerant strains of *Escherichia coli* O157:H7 and *L. monocytogenes*.

Methods: *Listeria monocytogenes* Scott A and the barotolerant OSY-328 strain, as well as two *E. coli* O157:H7 strains, EDL-933 and its barotolerant mutant, OSY-ASM, were tested. TBHQ (50 ppm, dissolved in dimethyl sulfoxide, DMSO) was added to cell suspensions (10^9 CFU/ml) in phosphate buffer (0.1 M, pH 7.0). Samples were pressurized at 200 to 500 MPa (23 ± 2°C holding temperature) for 1 min, plated on tryptose agar and incubated at 35°C for 48 h to enumerate the survivors.

Results: The UHP-TBHQ combination resulted in synergistic inactivation of both pathogens, with different degrees of lethality among strains. A theshold pressure should be achieved before bacteria are sensitized by TBHQ. This pressure lethality theshold for the combination treatment, was lower for *E. coli* O157:H7 (≥ 200 MPa) than for *L. monocytogenes* (> 300 MPa). Compared to *L. monocytogenes*, *E. coli* O157:H7 strains were extremely sensitive to the UHP-TBHQ treatment. Interestingly, a control treatment involving UHP-DMSO combination consistently resulted in higher inactivation than that achieved by UHP alone, against all strains tested. However, sensitization of pathogens to UHP by the additives was noticeably greater for TBHQ than DMSO.

Significance: Differences between these two pathogens in sensitivities to UHP, UHP-DMSO, or UHP-TBHQ treatment may be attributed to discrepancies in their cell envelope structure or physiological functions.

P5-34 Determination of Cell Numbers of *Clostridium botulinum* at Which Toxin is Detectable in Broth and Foods

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Environmental factors affecting *C. botulinum* spore germination, growth and toxin production are well documented. However, the number of *C. botulinum* vegetative cells needed to produce detectable botulinum toxin in broth or foods is unknown. The objective of this study was to determine the vegetative cell numbers of *C. botulinum* at which levels of *botulinum* toxin detectable by the mouse bioassay are produced in broth and selected foods. Spores of two strains each of proteolytic or non-proteolytic *C. botulinum* strains were inoculated into Trypticase-peptone-glucose-yeast broth (TPGY) or mashed potatoes for proteolytic strains and canned tuna for non-proteolytic strains. Inoculated samples were

assayed for *C. botulinum* cell numbers and botulinum toxin production every hour (in broth) or every 2 h (in foods) near toxin production times as determined previously in preliminary studies. Results revealed that *C. botulinum* cell numbers were at 6 to 8 log CFU/ml or g when toxin was first detected. Spore inoculation level (10 v. 1000 spores/ml or g) had little influence on cell numbers when toxin was initially detectable; however, a longer incubation time was needed for spores in the low level inoculum to germinate, grow, and produce detectable toxin. Incubation temperatures (31°C vs. 12° or 15°C) had little influence on cell numbers when toxin was first detected. A longer incubation time was needed at low temperatures to reach detectable toxicity. Proteolytic *C. botulinum* cell numbers were higher in broth than in foods when toxin was initially detected. Longer incubation times were needed for foods than broth to reach detectable toxicity for both types of *C. botulinum* strains. Non-proteolytic strains produced toxin at earlier sampling times than proteolytic strains, both in broth and foods.

P5-35 Enterotoxigenic Profile of *Bacillus cereus* Strains from Food Origin

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Bacillus cereus is an environmentally ubiquitous microorganism recognized as a foodborne hazard. In Brazil, the incidence of *B. cereus* outbreaks is unknown and there is little information about its occurence in food. This pathogen causes two distinct types of toxin-mediated foodborne illness, known as the diarrheal and emetic syndromes. *B. cereus* can cause diarrhea by producing heat-labile enterotoxins during growth of vegetative cells in the small intestine. Four different enterotoxins have been characterized as diarrheal: two protein complexes, hemolysin BL (HBL) and nonhemolytic enterotoxin (NHE) and two enterotoxic proteins, enterotoxin T and cytotoxin K. The complex HBL is composed of four proteins (B, B', L_1 and L_2) transcribed from the genes *hblC* (encoding L_2), *hblD* (encoding L1), *hblA* (encoding B) and *hblB* (encoding B'). NHE complex is produced by *nheA*, *nheB* and *nheC*, which encode the proteins *NheA*, *NheB* and *NheC*, respectively. In this study, the enterotoxin production capacity of 135 strains of *B. cereus* isolated from food products was evaluated using Duopath®; Cereus Enterotoxins (Merck), that uses genes target proteins hblC and nheB. All strains were also tested for the presence of the genes *hbl* (A, B, C and D) and *nhe* (*A*, *B* and *C*) using PCR. HBL was detected in 93 (68.9%) strains and NHE in 134 (99.3%) using Duopath®. The genes *hblA*, *B*, *C* and *D* were found in 53.3%, 75.6%, 38.5% and 91.9% of the strains, respectively, while the genes *nheA*, *B* and *C* were present in 68.9%, 47.4% and 97.8% of the strains, respectively. Some differences were found in the genetic and toxigenic potential of *B. cereus*, indicating the presence of strains that have the genes but do not express them. In summary, the novel Duopath® has been proven to be reliable and very easy-to-use test to rapidly detect and analyze enterotoxigenic *B. cereus* from food. Acknowledgment: Merck KGaA.

P5-36 Resistance Patterns of *Staphylococcus aureus* 8325-4 to Various Stress Conditions and Pathogenicity Profiles on HEp-2 Cell Lines

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Staphylococcus aureus is an important major pathogen causing a diverse array of threatening diseases worldwide. It has resistance toward various stresses and was shown to adhere to and invade various host cells in vitro. This study was conducted to understand resistance patterns of *S. aureus* to various stresses and pathogenicity profiles the in the absence of fibronectin in host cells. *S. aureus* 8325-4 was subjected to various stresses by treatment with heat, cold, acid, hydrogen peroxide and sodium chloride. Stress resistance was analyzed by quantifying the population reduction and protein expression profiles by 2-D SDS-PAGE. Human larynx epithelial (HEp-2) cells were used for bacterial adhesion, and internalization. Percentage of HEp-2 cell lysis was measured using the lactate dehydrogenase (LDH) assay; DNA fragmentation assay was used to determine the ability of treated *Staphylococcus aureus* to cause apoptosis, and morphological changes were assessed by light microscopy. Stress resistance was highest to heat (log 0.14) and lowest to H_2O_2 (log 2.6). Approximately 292 proteins could be identified by 2-D SDS-PAGE, having acidic proteins in higher ratio. Adhesion and invasion was significantly increased in H_2O_2 (log 6.49), acid (log 5.46) and heat (log 5.32) stresses, but numerically higher in cold and NaCl stresses. Varying LDH profiles were observed in stress treatments, but after 24 h, NaCl stress had the highest LDH release (34.8%) and heat stress had the lowest (18%). No apoptosis was observed in HEp-2 cells by DNA fragmentation and morphological changes. This study concludes that multiple factors of bacteria and host cells may be involved in resistence patterns. Further research on interacting proteins other than fibronectin and fibronectin binding protein is required in order to understand the host-microbe interactions of *Staphylococcus aureus*.

P5-37 Effect of Metabolic Enzymes on Amylopectin Content and Infectivity of *Cryptosporidium parvum*

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Introduction: Amylopectin granules in Apicomplexan protozoa could be used as an energy source to allow survival in the environment, allow latent stages to excyst, release infective stages, and invade host cells to continue their life cycle.

Purpose: The objective of this research was to determine if parasite glycolytic enzymes: (alpha-amylase, amyloglucosidase, enolase, lactate dehydroge-

nase, phosphorylase) could be used to decrease amylopectin stores and subsequently infectivity of *Cryptosporidium parvum*. In addition, glycolytic enzymes and substrates (glucose, glucose-1-phosphate, glycogen synthase) were investigated to determine if they could increase amylopectin stores and infectivity.

Methods: Oocysts of *Cryptosporidium parvum* were suspended in 1 mg/ml glycolytic enzymes or substrates (except glucose – 0.05 M and glycogen synthase – 1 U/ml) and electroporated. The oocysts were incubated at 37°C for one hour and were transferred to HCT-8 cells for infection to measure infectivity. Real-time PCR, immunohistochemistry, and excystation assays were performed to determine the effect of enzymes on infectivity/excystation, and treatment effects were compared by analysis of variance.

Results: Alpha amylase and amyloglucosidase had the greatest impact on amylopectin and reduced infectivity by 99.6% and 99.7%, respectively ($P < 0.05$). Amylopectin reduction was correlated with a decrease in infectivity and excystation of fresh oocysts but did not significantly decrease infectivity in stored oocysts. When enzymes/substrates were used to increase amylopectin, glycogen synthase had little effect, but glucose and glucose-1-phosphate significantly increased amylopectin, excystation, and infectivity.

Significance: Amylopectin may stimulate *C. parvum* infectivity due to excystation and release of infective sporozoites, but additional energy sources may be present in dormant oocysts. Further research is needed to clarify their role in the processes. This work may lead to further research on enzymes that can increase or decrease amylopectin as a control/drug target.

P5-38 The Potential for Pathogen Cross Contamination of Foods with Gloved Hands: Experiments with Feline Calicivirus as a Surrogate for Human Enteric Viruses

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Introduction: Because the United States Food Code (§3-301.11) prohibits bare hand contact of Ready-to-Eat foods to prevent pathogen transmission, the food industry has adopted the use of gloves as barrier protection. Although this may eliminate the risk of spread of pathogens through bare hands, the possibility of cross contamination of foods via gloved hands essentially remains unexplored.

Purpose: To determine the potential of gloved hands in the cross contamination of food contact surfaces, using a quantitative and standardized test protocol.

Methods: Each disk (1 cm diameter) of brushed stainless steel, representing hard food contact surfaces, received 10 µL (~1,000 PFU) of feline calicivirus (FCV) in a soil load. The inoculum was touched for 10 s at a pressure of 0.5 kg/cm^2 with either a bare or gloved fingerpad while the inoculum was still wet or after it had been allowed to become visibly dry. After a time lag of 10 s, fingerpads were either eluted or placed in contact with a sterile metal disk at the pressure and contact time already mentioned and eluted. The extent of acquisition and transfer was calculated, using plaque assays.

Results: Neither bare nor gloved fingerpads transferred any detectable levels of FCV from the disks with dried inocula. In contrast, both gloved and bare fingerpads could transfer up to 100 PFU to a clean disk from one with still wet inocula.

Significance: While gloving by foodhandlers is essential, gloves themselves could readily acquire, retain and transfer pathogens. Our testing, closely simulating interactions between hands and settings where foods are handled, further reinforce this. The findings have direct relevance to good food manufacturing and handling practices and to the education and training of foodhandlers.

P5-39 Construction of Internal Amplification Control and RNA Standard for Detection of Norovirus by Quantitative Real-time RT-PCR

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For reliable quantitative detection of foodborne pathogens by use of molecular amplification methods, a synthetic nucleic acid standard and an internal amplification control (IAC) must be designed. The former is used to standardize the assay, while the latter is critical for evaluating potential false negative results caused by matrix-associated inhibitors. This study reports the construction of a synthetic RNA standard and an IAC to be used in quantitative real-time RT-PCR (qRT-PCR) for the detection of the genogroup (GII) noroviruses (NoV), important agents of acute non-bacterial gastroenteritis. A previously reported qRT-PCR method (using primers JJV2F and COG2R) which targeted the highly conserved ORF1-ORF2 junction of the genome was used. This method produced an 87 bp product that was detected with an internal probe (RING2-TP) labeled with 6-carboxy-fluorescein (FAM) in a TaqMan format. The synthetic RNA standard (398 bp) contained the target region and was produced by RT-PCR amplification, using a forward primer labeled with the T7 promoter for production of the standard by in-vitro transcription. The IAC (230 bp) was designed by introducing diagnostic primer sites as 5' over-hanging ends of the NoV GII amplification primers with complementary 3' ends to plasmid pUC19. This produced a DNA fragment which could be amplified with the assay primers, but the rest of the sequence corresponded to pUC19 rather than to the target. The probe for the IAC was labeled with 6-carboxy—hexachloro-fluorescein (TET). Based on the RNA standard, the assay detection limit was < 13 RNA copies per reaction with a quantitative linear detection range from 101–105 copies. The IAC could be incorporated into the qRT-PCR reaction without loss of the detection sensitivity at an optimal concentration of 200 copies per reaction. The method performs well for

the detection of GII NoV in naturally and artificially contaminated clinical and food specimens. The approach provides a molecular means of quantitative detection of the GII NoV while simultaneously monitoring assay performance.

P5-40 Quantitative Evaluation of Human Norovirus Persistence in Different Types of Water

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Norovirus (NoVs) are the most commonly identified foodborne pathogens causing acute gastroenteritis. It was clearly shown that several types of food commodities may be involved in the transmission of NoVs to human. More recently, water has caused several NoVs outbreaks. Human NoVs are not grown in cell culture, and the use of virus models has not completely explained the environmental survival of human NoVs. Real-time TaqMan is currently the most sensitive method to detect low viral concentration, and their application was explored in NoVs clinical samples and naturally contaminated shellfish. Furthermore, quantitative RT-PCR is a valuable tool for the detection of reduced infectivity. In this study, we evaluated the persistence of human NoV-GII in several spiked water samples, using TaqMan real-time Reverse Transcription-PCR and conventional Reverse Transcription-PCR. Mineral, tap, river and sewage treated water samples were inoculated with geno-group II NoVs at a final concentration of 100 RT-PCR detectable units and incubated at 4°C, 25°C and -20°C. Samples were taken after 30 and 100 days and analyzed for the presence of NoVs by one of the molecular methods previously mentioned. NoV was shown to persist for two months in mineral and tap water for all temperatures tested. However, no viruses were detected after 100 days. At 4°C and -20°C, viral inactivation was lower than at 25°C. Lower viral reduction was observed at 4°C in river and sewage treated water samples after 1 month. Our study clearly shows that NoVs can persist in water samples for over 2 months in different environmental cond-itions. We have also shown that molecular techniques based on real-time amplification and detection of target sequences are very reliable for the quantification of human NoV.

P5-41 Persistence of Hepatitis A Virus on Foods and Food Preparation Surfaces

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Human enteric viruses are increasingly recognized as the causative agents for a large proportion of foodborne disease worldwide. Of these, hepatitis A virus (HAV) is one of the most epidemiologically relevant foodborne viral pathogens. The ability of enteric viruses to survive and persist in foods and in the environment most likely contributes to their high degree of transmissibility. In an effort to further explore this hypothesis, we initiated a study to evaluate the environmental persistence of HAV on common food preparation surfaces (stainless steel, ceramic, and formica) and on representative food items (lettuce and sliced deli meat) over their anticipated shelf lives. Food items were artificially inoculated with a stock culture of cytopathic HAV strain HM-175 diluted 20% in reconstituted artificial feces, followed by storage for up to 2 weeks at refrigeration (4°C) and room (22 ± 2°C) temperatures. Surface coupons (stainless steel, ceramic and formica) were likewise inoculated and held at room temperature for up to 6 weeks. At defined time points the virus was eluted from the foods or surfaces, processed for virus concentration with the Pathatrix™ system in conjuction with positively charged magnetic beads, and assayed for detection of viral RNA by use of quantitative real-time reverse transcription (RT)-PCR. HAV was found to be quite stable for a two week storage period on foods, at both ambient and refrigeration temperatures. On environmental surfaces, there was a slow but pronounced 2–3 log reduction in virus titer for six weeks under ambient conditions, and the viral RNA remained detectable even at the end of the experimental period. The findings confirm that HAV is quite environmentally stable, a characteristic that likely contributes to its transmissibility by foodborne routes.

P5-42 Growth Phenotypes, Genotypes and Cold Adaptation Gene Expression Responses in Two *Listeria monocytogenes* Strains of Human and Food-processing Environment Origin

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Introduction: The ability of *L. monocytogenes* to colonize and proliferate in food environments at low temperatures poses a significant challenge to microbial control in food products. Therefore, an improved understanding of the growth and gene expression responses of *L. monocytogenes* strains in cold stress environments might provide some insights into the cold adaptation features of these microbes.

Purpose: To investigate the growth phenotypes and gene expression responses of two *L. monocytogenes* strains under cold stress induced by incubation at refrigeration temperature at 4°C.

Methods: Strain Lm 22/3A of human origin and Lm 760 from a food-processing environment were comparatively evaluated in terms of:

(i) Growth phenotypes at 4°C
(ii) Genotypes, using PFGE, REP and ERIC PCR, and DNA sequencing
(iii) Cold adaptation gene expression responses

Results: Compared to Lm 22/3A, the Lm 760 strain displayed superior cold stress adaptation and growth capacity at 4°C. Despite clear differences in cold adaptation phenotypes, there were no detectable genetic differences between the two strains. Furthermore the two strains surprisingly displayed similar cold stress dependent expression induction of several cold adaptation gene targets (*sigB*, *betL*, *sigL*, *cspLA*, *cspLB*, *CspD* and *bglG* genes). While in contrast to the poor cold adaptation phenotype, cells of the Lm 22/3A strain displayed an even better induction of membrane based cold adaptation transporter proteins

encoded by the *gbuA* and *oppA* genes, the more cold resistant Lm 760 strain, on the other hand, mounted stronger induction of important cold adaptation genes *opuCB*, *fri*, *trxB* and *pgpH* at 4°C compared to the Lm 22/3A.

Significance: These data suggest that better expression levels of *opuCB*, *fri*, *trxB* and *pgpH* genes at 4°C in Lm 760 may confer cold stress adaptation advantages though their increased functional capacity under cold stress in this strain compared to Lm 22/3A.

P5-43 Growth Characteristics of *Listeria monocytogenes*, *Listeria welshimeri* and *Listeria innocua* Strains in Broth Cultures and a Sliced Bologna-type Product at 4 and 7°C

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Introduction: The ability of *Listeria* organisms to grow at low temperatures presents a significant challenge to food safety, especially with regard to the foodborne pathogen *L. monocytogenes*. An improved understanding of the contribution of field strain diversity in terms of growth responses under food related environments is important for more realistic predictions of risks associated with food products that may become contaminated with different types of field strains.

Purpose: To evaluate the growth characteristics in a collection of field *Listeria* spp. strains of *L. monocytogenes*, *L. innocua* and *L. welshimeri* derived from an industrial meat processing plant.

Methods: The growth of 6 *L. monocytogenes*, 6 *L. welshimeri* and 5 *L. innocua* strains was analyzed in BHI broth cultures held at 4°C and 7°C. The growth potential in two of the *L. monocytogenes* strains was assessed with food challenge tests on regular formulation and potassium lactate treated bologna type products.

Results: Significant variations were detected in the growth of various field-derived strains of the different *Listeria* species. Some of the *L. monocytogenes* field strains displayed better tolerance of the cold stress environment characterized by short lag phases and faster proliferation to reach high colony counts much earlier. These discrepancies were more apparent at 4°C than at 7°C. These properties were also retained in one of the *L. monocytogenes* food strains in food challenge tests on sliced bologna type sausages.

Significance: These findings further emphasize the influence of previous history and strain diversity on subsequent growth of *L. monocytogenes* organisms under refrigeration temperatures. This stresses the need for more evaluation of field strains growth behavior and for incorporation of such information in relevant predictive growth models for *L. monocytogenes* risk assessment in naturally contaminated food products.

P5-44 Modeling the Influence of Antimicrobials on the Spoilage Microflora of Cooked, Cured Meat Product

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Introduction: Cooked, cured meat products are perishable, Ready-to-Eat (RTE) products. Their salt content (2%), pH values (6.2–6.4) and water activity (0.98–0.99) are not inhibitory to the growth of spoilage microorganisms. Lactic acid bacteria (LAB) have been identified as spoilage organisms in cured meat. Shelf-life prolongation of RTE foods is of paramount importance to the meat industry.

Purpose: To evaluate the feasability of adding sodium chloride (NaCl), sodium lactate (SL) and/or adding sodium diacetate (SdA) to the batter to decelerate LAB growth and to extend the product shelf life.

Methods: A model was developed by using the binary logistic regression method to predict the LAB growth limits in a sliced vacuum-packaged cooked, cured meat product as a function of temperature (0 to 12°C), NaCl (0.5 to 3.5%), SL (0 to 3%) and SdA (0 to 0.3%). Fractional factorial Box-Behnken design was used to reduce the experimental trials to 140. Kinetic behavior of LAB at 4, 8, 12 and 16°C and at 10, 90 and 100% (control) probability of growth was also studied. Antimicrobials were used during the commercial preparation of the batter. To predict the desired probabilities of growth, the concentrations of NaCl, SL and SdA were obtained from the logistic model.

Results: The logistic model demonstrated the optimum combination of antimicrobials to inhibit LAB growth and to avoid product technological issues that could be raised from their arbitrary/empirical addition (e.g., pH decrease below 6.0 [SdA] and salty taste [NaCl and SL]). Kinetic behavior results showed that the product shelf life might increase by 30–40 days.

Significance: The data suggest that addition of antimicrobials to the cooked, cured meat product may contribute to product shelf-life prolongation.

P5-45 Comparison of Hydrostatic and Hydrodynamic Pressure to Inactivate Foodborne Viruses

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Introduction: Viruses cause the majority of cases of foodborne illness in the United States. The lack of culturability of some foodborne viruses requires the use of alternate models, like non-pathogenic surrogates or bacteriophage, for inactivation studies. High pressure technology is currently being implemented in deli meat processing and may be an effective method to inactivate viruses in foods.

Purpose: The effect of high hydrostatic pressure (HHP) and hydrodynamic pressure (HDP), in combination with chemical treatments, was evaluated for inactivation of foodborne viruses, non-pathogenic surrogates, and bacteriophage in a pork sausage product.

Methods: One-inch pieces of pork sausage were dipped in 2% lactoferrin, 100 mM EDTA, or water, and then inoculated with either 50 µl of feline calicivirus (FCV), hepatitis A virus (HAV) or bacteriophage (MS2, f174, or T4) and dried. Each piece was packaged individually and subjected to pressure by either HDP at 10°C, HHP (500 MPa, 5 min, 4°C), or untreated. Viruses or bacteriophage were recovered by vortexing and assayed by $TCID_{50}$ using cell culture or plaque assay with *E. coli* as a host strain.

Results: An average of 3.2 and 0.8 log HAV and 2.1 and 1.0 log FCV was inactivated by HHP and HDP, respectively. There was no significant difference in virus inactivation based on dipping in lactoferrin, EDTA, or water. There were no differences in inactivation of bacteriophage due to pressure treatments. HHP reduced f174 by 1.9 and 1.0 log PFU/g sausage after dipping in water and lactoferrin, respectively. No other combination of pressure and chemical treatments reduced bacteriophage titers by more than 1 log PFU/g sausage.

Significance: This study is the first to directly compare the inactivation of viruses and bacteriophage by two pressure processes (HHP and HDP). Inoculation of viruses and bacteriophage on a meat product may have protected them from higher levels of inactivation by pressure treatments.

P5-46 DSC A High Pressure Processing Inactivation Model for Hepatitis A Virus

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Introduction: Hepatitis A virus (HAV) causes acute infectious hepatitis in humans and has been implicated in a number of widespread outbreaks, particularly from the consumption of raw shellfish (e.g., oysters). The commercial success of applying high pressure processing (HPP) to improve food safety and quality while extending shelf life has generated interest in the effectiveness of HPP in eliminating viral contamination from shellfish.

Purpose: The purpose of this study was to develop an HPP inactivation model for HAV and evaluate the model in oyster meat artificially inoculated with HAV.

Methods: HAV suspended in buffered media containing either 15 or 30 parts per thousand (ppt) NaCl was subjected to pressures between 300 and 500 MPa at ambient temperature (approximately 18–20°C) for treatment times between 60 s to 600 s. Weibull and log-linear models were fitted to inactivation data, and the most appropriate model was evaluated by treating HAV artificially inoculated into Pacific oyster *(Crassostrea gigas)* homogenate, adjusted to 15 or 30 ppt NaCl, with HPP.

Results: Greater sensitivity to HPP was observed at 15 ppt NaCl than at 30 ppt NaCl. HAV was undetectable (3.5-log_{10} $TCID_{50}$/ml reduction to <1.47–$TCID_{50}$/ml) within 300 s and 360 s treatment at 500 MPa at 15 ppt and 30 ppt, respectively.

The Weibull model provided the better fit than the log-linear model to inactivation data for individual pressure/salinity treatments, but the fitted curve shapes were qualitatively inconsistent between treatments making interpolation between pressures difficult and unreliable. Assuming log-linear inactivation enabled a model to be developed that could describe the entire data set including interpolation between specific treatment conditions. The log-linear model was evaluated using HAV-inoculated oyster homogenates, providing generally fail-safe predictions at pressures ≥375 MPa.

Significance: The log-linear model developed may be useful for oyster processors intending to use HPP as an intervention process.

P5-47 Inactivation of *Cyclospora* Surrogate on Produce by Two Nonthermal Treatments

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Introduction: The protozoa *Cyclospora cayetanensis* has caused many cases of gastroenteritis in humans. Of specific interest are the cases in the US and Canada associated with contaminated raspberries and basil. *Cyclospora* infects only humans, making it difficult to obtain and a laboratory health hazard; therefore, the related poultry protozoa *Eimeria acervulina* was used as a surrogate.

Purpose: Ultraviolet light (UV) and high hydrostatic pressure (HHP) are both safe technologies with potential for use on raspberries and basil in packing houses, in the field, and in retail. Their effectiveness on *E. acervulina* oocyst inactivation was assessed by use of a live bird assay.

Methods: Raspberries and basil were individually inoculated with sporulated *E. acervulina* oocysts at a high (10^6) and low (10^4) dose. Inoculated and control produce were treated with UV (up to 160 mW/cm^2) or HHP (550 MPa). Oocysts were recovered from produce and fed to 3 wk old Sex-Sal male chickens, which were scored for weight gain, oocyst shedding, and lesions. Control birds were sham inoculated.

Results: Oocysts exhibited enhanced excystation on raspberries but not on basil, perhaps an indication of enhanced infectivity. Oocysts on raspberries treated with 160 mW/cm^2 showed reduced infection, while oocysts alone yielded no lesions. These birds all had healthy weight gain but shed oocysts. HPP treatment (550 MPa, 2 min, 4°C) of basil completely inactivated oocysts, and birds fed treated raspberries showed no clinical signs of infection. The inoculum level is important, as birds fed raspberries and basil inoculated with 10^4 oocysts treated with HHP were completely asymptomatic and those fed basil with 10^6 oocysts treated with HHP shed oocysts.

Significance: This study suggests that UV light and HHP may be ways to treat produce at risk for *Cyclospora* contamination. Both treatments yielded healthy animals; however, HHP was more effective, as observed in studies using high inoculums.

P5-48 DSC Putative Innovations in Extraction of Genomic DNA of Lactic Acid Bacteria

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Assessment of safety criteria encompassing bacteria at large — and probiotic bacteria in particular—includes screening for virulence factors, which includes DNA extraction as a crucial step. Cell wall lysis is a key stage therein; in the case of some Lactic Acid Bacteria (LAB), including probiotic ones, it may even become critical, likely because of the S-layer and O-acetylated MurNAc residues of their constituent peptidoglycans. This report encompasses a protocol for DNA extraction from LAB, especially from the genera *Lactobacillus* and *Bifidobacterium*, in which the combined effects of lysozyme and mutanolysin permit high concentrations of clean DNA to be attained in a short period of time. Twenty-two (potentially probiotic) LAB strains were tested in this study: 16 *Lactobacillus*, 5 *Bifidobacterium* and 1 *Lactococcus*. Upon extraction of DNA following the novel protocol, its concentration was measured, using an Ultraspec 2100 pro UV/Visible spectrophotometer; DNA was also observed via electrophoresis on 1% agarose gel, where bands were visualized with ethidium bromide under UV irradiation, which permitted one to conclude that the DNA obtained was clean. The average concentrations of DNA obtained were 4826 mg/ml for lactobacilli, 5204 mg/ml for bifidobacteria and 9082 mg/ml for *lactococcus*. The proposed method allows good extraction of genomic DNA, with high yields, and although the protocol does not include a purification step, the DNA obtained is essentially clean.

P5-49 Use of Molecular Typing for Classification of Environmental Isolates of *Penicillium* spp.

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Introduction: The penicillia are ubiquitous within the environment and have been isolated from a diverse range of food types in which they lead to product spoilage. Spoilage is one of the key issues faced by the food industry in terms of product wastage and its associated financial losses. To date, most of the molecular analysis of molds has been performed with clinically relevant organisms, and this study will analyze the use of DNA-based typing method for food related isolates.

Purpose: To evaluate a molecular-based typing method for *Penicillium* spp. food and environmental isolates.

Methods: DNA extracted from a selection of phenotypically related and unrelated *Penicillium* spp. isolates was amplified by use of a mold specific primer set. and the resulting products were resolved using chip-based electrophoresis. The fragment patterns generated were analyzed to determine the relatedness of isolates, using Pearson correlation.

Results: Data from this study demonstrated that molecular typing can be used to cluster isolates with similar phenotypic characteristics, giving similarity scores of 97.2–99.3%. The profiles showed a wide diversity among the penicillia, with significant differences in the fragment patterns, which was also reflected in the similarity scores, which were as low as 50.2%.

Significance: The results indicate that molecular typing has the potential to be used for *Penicillium* spp. isolated from foods and the processing environment.

P5-50 Detection and Enumeration of Microorganisms in Ready-to-Eat Foods, Ready-to-Cook Foods and Fresh-cut Produce in Korea

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This study was executed to investigate levels of total aerobic plate count (APC), coliforms and various pathogens in convenience foods such as Ready-to-Eat foods (RTE), ready-to-cook foods (RTC), and fresh-cut produce (FCP) in Korea. A total of 244 samples, consisting of 145 RTE samples, 39 RTC samples, and 60 FCP samples were purchased from supermarkets in six different provinces. The levels of APC in RTE varied between 1.9 ~7.8 log CFU/g, whereas the levels in RTC and FCP were in the range of 6.3 ~8.1 log CFU/g. Even though coliforms were not detected in approximately 50% of samples, the remaining samples were contaminated by coliforms up to 5 log CFU/g. *E. coli* was the most frequently detected microorganism in convenience foods, followed by *Staphylococcus aureus*. *Salmonella* spp. and *Listeria monocytogenes* were detected in only one sample and *Vibrio parahaemolyticus* was not detected in any samples. These results showed that convenience foods such as RTE, RTC, FCP could be contaminated by various pathogenic bacteria in Korea. Therefore, precautionary measures are necessary for consumer protection, such as improving the sanitary condition of the processing plants of RTE foods, RTC, and FCP foods in Korea.

P5-51 Survival of *Bifidobacterium adolescentis* 15703T in Uncoated Gelatin and Alginate-coated Gelatin Microspheres during Exposure to Simulated Gastrointestinal Conditions

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Survival of the fastidious probiotic bifidobacteria in food products and during exposure to the acidic upper part of the gastrointestinal tract can be problematic and negatively affect the delivery of these

health promoting bacteria to the consumer. In this study, uncoated gelatin and alginate-coated gelatin micro-spheres were produced to encapsulate the probiotic *Bifidobacterium adolescentis* 15703T in an attempt to enhance survival. Alginate coating of gelatin microspheres was obtained by two methods using cross linking of alginate by either external Ca^{+2} or internal Ca^{+2} diffusion methods. Microsphere sizes, encapsulation yields of cells and confocal laser microscopy images of coated and uncoated microspheres were obtained. The survival of free and encapsulated cells was determined in simulated gastric juice and during sequential incubation in simulated gastric and intestinal juices. Confocal laser microscopy revealed a thin uniform alginate coating along the periphery of the gelatin matrix. Microsphere sizes (49.0–53.1 μm) were consequently not significantly ($P > 0.05$) different while encapsulation yields ranged from 30–43%. After 120 min in simulated gastric juice, free cell counts decreased by 3.45 log (CFU/ml) units compared to decreases of 2.55, 1.75 and 1.21 log units for gelatin microencapsulated cells in uncoated, alginate-coated by internal and external Ca^{+2} sources, respectively. Sequential incubation in simulated intestinal juice after 60 min in simulated gastric juice resulted in the disintegration of the alginate coating at the neutral pH of the intestinal juice. Reduction in viable numbers of released bacteria indicated sensitivity to the presence of bile salts in the simulated intestinal juice. Alginate coated microspheres achieved higher surviving numbers of cells after 240 min in simulated intestinal juice, $10^{7.6}$ and $10^{7.4}$ CFU/ml by external and internal Ca^{+2} sources, respectively, while $10^{6.4}$ and $10^{6.7}$ CFU/ml were obtained for free cells and uncoated gelatin encapsulated cells, respectively. In conclusion, microencapsulation in alginate-coated gelatin microspheres significantly improved survival of *B. adolescentis* during exposure to simulated gastrointestinal conditions.

P5-52 pH Contribution in the Evaluation of Quorum Sensing in Milk

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Introduction: The occurrence of autoinducers (e.g., AHL) or autoinducer-like compounds in foods during storage and their potential role in spoilage have been only recently questioned in the literature. The contribution of Gram-negative bacteria in the spoilage of high pH products (e.g., meat, milk) is of great importance, and so far the role of the above mentioned substances is not fully understood.

Purpose: The current study aims to (1) make an initial qualitative assessment of raw, pasteurized and UHT milk, by correlating the levels of microflora in milk with the type of the AHLs detected in cell-free milk supernatants and to (2) provide further information regarding the behavior of AHLs and intrinsic factors that occur during storage. The influence of pH on the biological activity of AHLs was investigated.

Methods: The levels of total viable counts, *Enterobacteriaceae* and pseudomonads were assessed for raw, pasteurized and UHT milk, while the occurrence of Quorum sensings in cell-free milk supernatants (CFMS) was detected by use of *Agrobacterium tumefaciens* A136 and *Chomobacterium violaceum* CV026 reporter strains. The effect of pH on the stability of AHL-like compounds was also investigated in CFMSs and pure cell free cultures of *A. tumefaciens* KYC6 and *C. violaceum* 31532 producer strains.

Results: The *A. tumefaciens* but not the *C. violaceum* responded to CFMS in all milk types regardless of fat concentration and heat processing. The effect of the pH on Quorum Sensing compounds (e.g., AHL) was significant with both reporters and occurred only after the acidification of CFMS samples for a certain time period (ca 4 h).

Significance: By understanding the factors affecting the properties of certain AHLs compounds in food systems, specific autoinducer inhibitors could be used on food preservation.

P5-53 DSC Determination of Surrogate Organisms for *Listeria monocytogenes* Treated with Ultra-high Pressure and Pulsed-electric Field

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Ultra-high pressure (UHP) and pulsed-electric field (PEF) are promising alternative processing techniques that are being optimized to enhance safety and/or shelf stability of treated food. It is crucial to recognize the pathogens of concern (targeted pathogens) for food treated with these technologies. Additionally, it is equally important to identify low-risk surrogate microorganisms that can be used in lieu of the targeted pathogens in food processing facility. The objective of this study was to determine appropriate surrogate organisms for *Listeria monocytogenes* for UHP and PEF processing. Candidate surrogates tested include four *Lactobacillus* spp., a *Pediococcus* sp. and a *Listeria innocua* strain. Candidate surrogates and nine *L. monocytogenes* strains were treated with UHP and PEF and processing sensitivity of these microorganisms was determined. For UHP, strains were suspended in 0.1% NaCl or citrate-phosphate buffer (pH 7.0 or pH 4.5) and processed at 500 MPa with a holding time of 1 minute. For PEF, each strain was suspended in 0.1% NaCl or 50% acid whey and was individually processed with a continuous PEF apparatus at 25 kV/cm. Inactivation by PEF and UHP was strain-dependent and varied with media composition and pH. Pressure treatments of *L. monocytogenes* strains suspended in 0.1% NaCl inactivated 3.8 to 6.0 log CFU/ml, whereas treatment in citrate-phosphate buffer (pH 7.0) inactivated 0.3 to 2.0 log CFU/ml only. When PEF-processed in 0.1% NaCl at 23°C, *L. monocytogenes* populations decreased 0.7 -5.3 log CFU/ml. *Listeria monocytogenes* V7 and OSY-8578 were among the most resistant strains to both UHP and PEF treatments and would be ideal target organisms. *Listeria innocua* and *L. plantarum* were similar to the target organisms in resistance to UHP and PEF treatments and would be good surrogate organisms for both processes. In the case of UHP, a *Pediococcus* sp. was the most resistant bacterium tested, with < 0.25 log inactivation.

P5-54 Carriage of *Escherichia coli* O157 by European Starlings

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Introduction: The sources of novel strains of *Escherichia coli* O157 on closed dairy herds and on vegetable production sites are unknown. Wild birds, particularly European Starlings (*Sturnus vulgaris*), are known to frequent farms and can carry *E. coli* O157 and other foodborne bacterial pathogens.

Purpose: The objective of this study was to determine (1) the minimum infectious dose required for *E. coli* O157 to colonize starlings; (2) the duration of *E. coli* O157 shedding by starlings; and (3) the magnitude of *E. coli* O157 shedding by starlings.

Methods: Wild-caught European starlings were individually housed and orally inoculated with various doses of *E. coli* O157. *E. coli* O157 present in the excrement of individual birds was determined by quantitative culture methods.

Results: Birds that were given 10^4 or less CFU (n = 30) shed the organisms for less than three days, whereas birds dosed with 10^5 or more CFUs (n = 20) excreted the organisms for 10 days. The average magnitude of *E. coli* O157 among positive birds, 3 log CFU/g, did not differ with inoculum dose. The concentration of *E. coli* O157 in bird guano stored at room temperature decreased by 2-log units over a 2-week period. *E. coli* O157 was transferred between starlings and calves following cohabitation for one day.

Significance: European starlings can readily acquire *E. coli* O157 from bovine sources and subsequently serve as a vector for dissemination of this pathogen to other livestock operations and to crops.

P5-55 DSC Microbiological Analysis of United States Currency Collected from Food Service Establishments

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Introduction: Money changes hands frequently. Several studies have revealed the microbial contamination of both dollar bills and coins. The contaminated money handled by cashiers in food establishments may transmit foodborne pathogens to food products or consumers.

Purpose: This study determined the contamination levels of US dollar bills with potential foodborne pathogens such as *Escherichia coli*, *Salmonella*, *Bacillus cereus*, and *Staphylococcus aureus*, and their antibiotic susceptibility as well.

Methods: Ten $5 and $20 bills were collected from each of six locations (3 grocery stores and 3 restaurants). Bacterial enumeration was conducted using the pour-plate method. For specific pathogen detection, each sample was incubated in enrichment broth overnight at 35°C. A loopful of enrichment culture was streaked onto MacConkey agar, *Bacillus* selective agar, and Baird Parker agar with potassium tellurite and egg yolk for detection of *E. coli*/coliforms, *B. cereus*, and *S. aureus*, respectively. The standard FDA-BAM procedures were followed for *Salmonella* detection. All suspected pathogenic isolates were gram-stained, and they were then tested for antibiotic susceptibility on trypticase soy agar (TSA)/ciprofloxacin (4 μg/ml), TSA/tetracycline (16 μg/ml), and TSA/penicillin (4 μg/ml).

Results: The average total bacterial counts for $5 and $20 bills were 4.03×10^3 and 2.41×10^3/bill, respectively. *B. cereus*, *S. aureus*, *Salmonella*, and *E. coli*/coliforms were positive in 28.3, 31.6, 13.3, and 31.7% of $5 bills, respectively, and in 21.7, 30, 40, and 43.3% of $20 bills, respectively. Further antibiotic susceptibility tests revealed that about 58.3, 3.6, 0, and 1.5% of *B. cereus*, *S. aureus*, *Salmonella*, and *E. coli* isolates, respectively, were resistant to tetracycline. Over 90% of all above isolates were resistant to penicillin, but only *S. aureus* isolates showed resistance to ciprofloxacin.

Significance: Our data indicate that money serves as a source of microorganisms and can potentially aid in the transmission of harmful bacteria to food products and consumers.

P5-56 Microbiological Quality of Pork Meat from Mexican Supermarkets

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Introduction: Microbiological contamination of meat products is undesirable, and mishandling can occur at any point in the food chain. The presence or proliferation of any pathogen presents an important risk for the consumer.

Purpose: This study was conducted to investigate microbiological quality and the presence of several pathogens in retail fresh pork meat.

Methods: A total of one hundred and fifty samples of raw pork meat were purchased from local supermarkets in the city of Monterrey N.L. Mexico and the metropolitan area from January to November 2006. All samples were examined for mesophiles, total coliforms, fecal coliforms, molds and yeasts, and the presence of several pathogens, such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter* spp., *Escherichia coli* O157:H7, *Listeria* spp., *Yersinia* spp., *Shigella* spp. and *Clostridium perfringens*. The presence of *Salmonella* spp., *L. monocytogenes* and *S. aureus* in foods was determined by use of the Mexican Official Methods, whereas for the other bacteria, the Bacteriological Analytical Manual (BAM) protocols were used.

Results: More than 80% of the samples had less than 10^4 CFU/g mesophiles. Seventy-five percent of the samples had less than 10^3 CFU/g coliforms and 90% contained less than 10^3 CFU/g fecal coliforms. Eleven raw pork meat samples were contaminated with, *S. aureus* (7.3%), 15 had *Salmonella* spp. (10%), 6 had *Campylobacter* spp. (3.3%), 14 had *E. coli* O157:H7 (13.3%), 48 had *Listeria* spp. (32.6%), 38 had *Yersinia* spp. (25%), 3 had *Shigella* spp. (2%) and 24 had *Clostridium perfringens* (16%). Six samples were contaminated with 4 different pathogens.

Significance: Pathogens were present in a considerable number of commercial raw meat samples. If improper cooking or handling occurs, this could be a source of infection for consumers.

P5-57 Microbiological Evaluation of Infant Bottles Used for Feeding Powdered Formula: Implications for *Enterobacter sakazakii*

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Introduction: Microbial contamination of powdered infant formula (PIF) with bacteria such as *Enterobacter sakazakii* has been known to cause a relatively rare but often fatal infection in neonates. This has implications both for the quality assurance procedures during PIF manufacture and subsequent preparation and storage by end users; another potential source could be incorrectly cleaned bottles used to hold PIF.

Purpose: The aim of this study was to evaluate the cleanliness and microbial load of "in-use" bottles in relation to how they are cleaned and sterilized.

Methods: To establish the baseline microbial contamination of "used" plastic feeding bottles, 100 uncleaned bottles and 100 sterilized bottles used for feeding PIF to infants aged < 6 months with PIF were obtained from parents and nurseries. Each bottle was sampled in 4 sites (inner screwcap, bottle interior; bottle outer rim, teat interior) to determine aerobic plate counts (APC), *Enterobacteriaceae* and *S. aureus* counts and levels of residual adenosine triphosphate (ATP).

Results: APC counts (n = 400) from uncleaned bottles ranged from 4.0×10^2 to 2.9×10^5. Counts of $< 1.0 \times 10^2$ *Enterobacteriaceae* were isolated from < 7% of bottles and no *S. aureus* was identified. Microbial counts were variable according to sampled bottle site. ATP values for screwcap interiors were as high as 20,188 relative-light-units (RLUs), whereas outer rims attained a maximum of 2486 RLUs. Teat and screwcap interiors were the most heavily contaminated sites sampled. Data indicates that after use there was considerable microbial and organic soil remaining in the bottles. Of the bottles reportedly cleaned and sterilized by parents/nurseries, some had in excess of 7.5×10^4 APCs; no *Enterobacteriaceae* or *S. aureus* were detected; ATP values up to 1535 RLUs were obtained. Contamination was most frequently detected from the screwcap and teat interiors, whereas bottle interiors were least likely to be contaminated.

Significance: Results cumulatively indicate the presence of organic soiling and the potential for cross survival of bacteria between infant feeds. This highlights the need for education on effective bottle decontamination procedures.

P5-58 Microbial Analyses of Fungi in Cereal Bars

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Introduction: Cereal bars are products accepted and consumed worldwide by individuals of different ages for the most diverse purposes. However, due to the high level of cereals in its composition, fungi can grow and produce mycotoxins that can be carcinogenic.

Purpose: To verify the presence of fungi in reduced-calorie and regular cereal bars and indentify any fungi in the samples.

Methods: The experiments were carried out in duplicates using 20 cereal bars (10 reduced calorie and 10 light) of 10 different brands from the Brazilian market. The samples were diluted and incubated for 3 to 5 days at 25°C in potato dextrose acidified agar (PDA) medium. After incubation, counting of the CFU/g and identification though the morphophysiological analysis of each isolated colony of fungus, using classic microbiological techniques of identification, were performed.

Results: It was verified that 4 samples of 10 for both types of bar (reduced calorie and regular) presented some contamination by six different species of fungi. The contaminations varied from 1.7 to 3.2 log CFU/g and from 2 to 3.9 log CFU/g for reduced calorie and regular cereal bars, respectively. The fungi *Penicillium* sp. and *Aspergillus* sp. were identified in 50% and 12.5% of the contaminated samples, respectively, while the yeast *Candida* sp. was found in 37.5% of the samples. Also identified were *Rhizopus* spp., *Cladosporium* spp. and *Mucor* spp.

Significance: The detection of six different species of fungi in cereal bars indicates that improvement in the quality control of these foods is necessary, mainly because of the presence of *Aspergillus* spp. and *Penicillium* spp., which, because of their ability to produce mycotoxins have carcinogenic potential.

P5-59 DSC Incidence of *Listeria* in Rural Home Environments with and without Ruminant Animals

MAWILL RODRIGUEZ-MARVAL, Ifigenia Geornaras, Patricia Kendall, Lydia Medeiros, Jeff LeJeune, and John N. Sofos, Colorado State University, 9 Animal Science Bldg., Fort Collins, CO 80523-1171, USA

Introduction: A major portion of foodborne disease cases may occur at home, but research examining the home environment as a source of pathogens, such as *Listeria monocytogenes*, is limited. It would be useful to evaluate differences in pathogen prevalence between homes with and without ruminants, since ruminants may be *L. monocytogenes* carriers. Such knowledge could be used to provide consumers with recommendations for proper cleaning and food handling.

Purpose: The objective of this study was to determine *L. monocytogenes* incidence in household environments of residents with/without direct contact with ruminants or their environment, and to correlate its presence with household type and environmental variables.

Methods: Fifty-four Colorado households (27 each with and without ruminants) were visited every 3–4 weeks in January–June 2006 (four visits each). During the first visit, observations of kitchen and refrigerator cleanliness were recorded, and participants were surveyed about eating and food-handling habits. At each visit, environmental (refrigerator,

P5-54 Carriage of *Escherichia coli* O157 by European Starlings

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Introduction: The sources of novel strains of *Escherichia coli* O157 on closed dairy herds and on vegetable production sites are unknown. Wild birds, particularly European Starlings (*Sturnus vulgaris*), are known to frequent farms and can carry *E. coli* O157 and other foodborne bacterial pathogens.

Purpose: The objective of this study was to determine (1) the minimum infectious dose required for *E. coli* O157 to colonize starlings; (2) the duration of *E. coli* O157 shedding by starlings; and (3) the magnitude of *E. coli* O157 shedding by starlings.

Methods: Wild-caught European starlings were individually housed and orally inoculated with various doses of *E. coli* O157. *E. coli* O157 present in the excrement of individual birds was determined by quantitative culture methods.

Results: Birds that were given 10^4 or less CFU (n = 30) shed the organisms for less than three days, whereas birds dosed with 10^5 or more CFUs (n = 20) excreted the organisms for 10 days. The average magnitude of *E. coli* O157 among positive birds, 3 log CFU/g, did not differ with inoculum dose. The concentration of *E. coli* O157 in bird guano stored at room temperature decreased by 2-log units over a 2-week period. *E. coli* O157 was transferred between starlings and calves following cohabitation for one day.

Significance: European starlings can readily acquire *E. coli* O157 from bovine sources and subsequently serve as a vector for dissemination of this pathogen to other livestock operations and to crops.

P5-55 DSC Microbiological Analysis of United States Currency Collected from Food Service Establishments

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Introduction: Money changes hands frequently. Several studies have revealed the microbial contamination of both dollar bills and coins. The contaminated money handled by cashiers in food establishments may transmit foodborne pathogens to food products or consumers.

Purpose: This study determined the contamination levels of US dollar bills with potential foodborne pathogens such as *Escherichia coli*, *Salmonella*, *Bacillus cereus*, and *Staphylococcus aureus*, and their antibiotic susceptibility as well.

Methods: Ten $5 and $20 bills were collected from each of six locations (3 grocery stores and 3 restaurants). Bacterial enumeration was conducted using the pour-plate method. For specific pathogen detection, each sample was incubated in enrichment broth overnight at 35°C. A loopful of enrichment culture was streaked onto MacConkey agar, *Bacillus* selective agar, and Baird Parker agar with potassium tellurite and egg yolk for detection of *E. coli*/coliforms, *B. cereus*, and *S. aureus*, respectively. The standard FDA-BAM procedures were followed for *Salmonella* detection. All suspected pathogenic isolates were gram-stained, and they were then tested for antibiotic susceptibility on trypticase soy agar (TSA)/ciprofloxacin (4 μg/ml), TSA/tetracycline (16 μg/ml), and TSA/penicillin (4 μg/ml).

Results: The average total bacterial counts for $5 and $20 bills were 4.03×10^3 and 2.41×10^3/bill, respectively. *B. cereus*, *S. aureus*, *Salmonella*, and *E. coli*/coliforms were positive in 28.3, 31.6, 13.3, and 31.7% of $5 bills, respectively, and in 21.7, 30, 40, and 43.3% of $20 bills, respectively. Further antibiotic susceptibility tests revealed that about 58.3, 3.6, 0, and 1.5% of *B. cereus*, *S. aureus*, *Salmonella*, and *E. coli* isolates, respectively, were resistant to tetracycline. Over 90% of all above isolates were resistant to penicillin, but only *S. aureus* isolates showed resistance to ciprofloxacin.

Significance: Our data indicate that money serves as a source of microorganisms and can potentially aid in the transmission of harmful bacteria to food products and consumers.

P5-56 Microbiological Quality of Pork Meat from Mexican Supermarkets

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Introduction: Microbiological contamination of meat products is undesirable, and mishandling can occur at any point in the food chain. The presence or proliferation of any pathogen presents an important risk for the consumer.

Purpose: This study was conducted to investigate microbiological quality and the presence of several pathogens in retail fresh pork meat.

Methods: A total of one hundred and fifty samples of raw pork meat were purchased from local supermarkets in the city of Monterrey N.L. Mexico and the metropolitan area from January to November 2006. All samples were examined for mesophiles, total coliforms, fecal coliforms, molds and yeasts, and the presence of several pathogens, such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter* spp., *Escherichia coli* O157:H7, *Listeria* spp., *Yersinia* spp., *Shigella* spp. and *Clostridium perfringens*. The presence of *Salmonella* spp., *L. monocytogenes* and *S. aureus* in foods was determined by use of the Mexican Official Methods, whereas for the other bacteria, the Bacteriological Analytical Manual (BAM) protocols were used.

Results: More than 80% of the samples had less than 10^4 CFU/g mesophiles. Seventy-five percent of the samples had less than 10^3 CFU/g coliforms and 90% contained less than 10^3 CFU/g fecal coliforms. Eleven raw pork meat samples were contaminated with, *S. aureus* (7.3%), 15 had *Salmonella* spp. (10%), 6 had *Campylobacter* spp. (3.3%), 14 had *E. coli* O157:H7 (13.3%), 48 had *Listeria* spp. (32.6%), 38 had *Yersinia* spp. (25%), 3 had *Shigella* spp. (2%) and 24 had *Clostridium perfringens* (16%). Six samples were contaminated with 4 different pathogens.

Significance: Pathogens were present in a considerable number of commercial raw meat samples. If improper cooking or handling occurs, this could be a source of infection for consumers.

P5-57 Microbiological Evaluation of Infant Bottles Used for Feeding Powdered Formula: Implications for *Enterobacter sakazakii*

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Introduction: Microbial contamination of powdered infant formula (PIF) with bacteria such as *Enterobacter sakazakii* has been known to cause a relatively rare but often fatal infection in neonates. This has implications both for the quality assurance procedures during PIF manufacture and subsequent preparation and storage by end users; another potential source could be incorrectly cleaned bottles used to hold PIF.

Purpose: The aim of this study was to evaluate the cleanliness and microbial load of "in-use" bottles in relation to how they are cleaned and sterilized.

Methods: To establish the baseline microbial contamination of "used" plastic feeding bottles, 100 uncleaned bottles and 100 sterilized bottles used for feeding PIF to infants aged < 6 months with PIF were obtained from parents and nurseries. Each bottle was sampled in 4 sites (inner screwcap, bottle interior; bottle outer rim, teat interior) to determine aerobic plate counts (APC), *Enterobacteriaceae* and *S. aureus* counts and levels of residual adenosine triphosphate (ATP).

Results: APC counts (n = 400) from uncleaned bottles ranged from 4.0×10^2 to 2.9×10^5. Counts of $< 1.0 \times 10^2$ *Enterobacteriaceae* were isolated from < 7% of bottles and no *S. aureus* was identified. Microbial counts were variable according to sampled bottle site. ATP values for screwcap interiors were as high as 20,188 relative-light-units (RLUs), whereas outer rims attained a maximum of 2486 RLUs. Teat and screwcap interiors were the most heavily contaminated sites sampled. Data indicates that after use there was considerable microbial and organic soil remaining in the bottles. Of the bottles reportedly cleaned and sterilized by parents/nurseries, some had in excess of 7.5×10^4 APCs; no *Enterobacteriaceae* or *S. aureus* were detected; ATP values up to 1535 RLUs were obtained. Contamination was most frequently detected from the screwcap and teat interiors, whereas bottle interiors were least likely to be contaminated.

Significance: Results cumulatively indicate the presence of organic soiling and the potential for cross survival of bacteria between infant feeds. This highlights the need for education on effective bottle decontamination procedures.

P5-58 Microbial Analyses of Fungi in Cereal Bars

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Introduction: Cereal bars are products accepted and consumed worldwide by individuals of different ages for the most diverse purposes. However, due to the high level of cereals in its composition, fungi can grow and produce mycotoxins that can be carcinogenic.

Purpose: To verify the presence of fungi in reduced-calorie and regular cereal bars and indentify any fungi in the samples.

Methods: The experiments were carried out in duplicates using 20 cereal bars (10 reduced calorie and 10 light) of 10 different brands from the Brazilian market. The samples were diluted and incubated for 3 to 5 days at 25°C in potato dextrose acidified agar (PDA) medium. After incubation, counting of the CFU/g and identification though the morphophysiological analysis of each isolated colony of fungus, using classic microbiological techniques of identification, were performed.

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Significance: The detection of six different species of fungi in cereal bars indicates that improvement in the quality control of these foods is necessary, mainly because of the presence of *Aspergillus* spp. and *Penicillium* spp., which, because of their ability to produce mycotoxins have carcinogenic potential.

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MAWILL RODRIGUEZ-MARVAL, Ifigenia Geornaras, Patricia Kendall, Lydia Medeiros, Jeff LeJeune, and John N. Sofos, Colorado State University, 9 Animal Science Bldg., Fort Collins, CO 80523-1171, USA

Introduction: A major portion of foodborne disease cases may occur at home, but research examining the home environment as a source of pathogens, such as *Listeria monocytogenes*, is limited. It would be useful to evaluate differences in pathogen prevalence between homes with and without ruminants, since ruminants may be *L. monocytogenes* carriers. Such knowledge could be used to provide consumers with recommendations for proper cleaning and food handling.

Purpose: The objective of this study was to determine *L. monocytogenes* incidence in household environments of residents with/without direct contact with ruminants or their environment, and to correlate its presence with household type and environmental variables.

Methods: Fifty-four Colorado households (27 each with and without ruminants) were visited every 3–4 weeks in January–June 2006 (four visits each). During the first visit, observations of kitchen and refrigerator cleanliness were recorded, and participants were surveyed about eating and food-handling habits. At each visit, environmental (refrigerator,

kitchen sink and countertop, washing machine, and shoes), food, and fecal samples (from ruminants and household members) were collected and analyzed for *L. monocytogenes* presence, using the US Dept. of Agriculture Food Safety and Inspection Service procedure (one sample/type/visit). Statistical analysis following the PROCFREC procedure in SAS® was used to correlate environmental variables with the probability of *L. monocytogenes* presence.

Results: *L. monocytogenes* was isolated from all types of samples except human stools. Forty-four percent (12/27) of non-ruminant households had at least one *Listeria* spp. positive sample, compared to 37% (10/27) of ruminant households. No difference ($P > 0.1$) was found in incidence between ruminant and non-ruminant households, and only one household of each type had positive samples on multiple visits. *L. monocytogenes* incidence in food and ruminant fecal samples was 1.6% (10/642) and 8.3% (9/108), respectively.

Significance: Since the household environment is a potential source of *L. monocytogenes* contamination, consumers should be informed/educated in this regard to prevent infection with this pathogen.

P5-60 Microbiological Quality of Selected Foods from United Kingdom Retail Premises, with a Focus on *Listeria monocytogenes*

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Introduction: Although listeriosis is a rare disease in the UK, a rise in the number of listeriosis cases in the UK has occurred over the last five years, particularly in people over 60 years. This is a cause for concern in view of the increased risk posed to this vulnerable group and the severity of the disease.

Purpose: The reason for the increase in listeriosis is unclear. In an attempt to understand this increase, an on-going study that focused on Ready-to-Eat foods that have been linked to the recent rise and/or case food histories was initiated from May 2006 onwards, with the aim to investigate the microbiological quality of these products.

Methods: Ready-to-Eat foods (sliced meats, sandwiches, cheeses, butter, probiotic drinks, and confectionery products containing cream) were sampled, based upon a Shopping Basket approach, from retail premises and examined for presence and levels of *Listeria* spp., including *Listeria monocytogenes*.

Results: 1894 samples were examined during the first six months (May to October 2006), and 3% and 6% of samples contained *L. monocytogenes* and other *Listeria* spp. respectively. 0.3% of samples failed EC legal food safety criteria due to the presence of *L. monocytogenes* in excess of 100 CFU/g (range 10^2–10^4 CFU/g); all of these samples were sliced pre-packed meats. A further 2 (0.1%) samples were unsatisfactory due to high levels of *Listeria* spp. (*L. welshimeri* 1.8×10^2 CFU/g, *L. innocua* 3.7×10^2 CFU/g).

Significance: Results from the first six months of sampling indicates that the control of *L. monocytogenes* in foods consumed by vulnerable groups is essential to minimize the potential for this bacterium to be present and multiply, particularly in pre-packed sliced meats at the point of consumption, to levels hazardous to health.

P5-61 DSC *Aspergillus flavus* and *Penicillium* spp. Associated with Musty Off Flavors in Navy Beans

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Introduction: Musty off flavors and odors associated with navy bean products are undesirable. Geosmin and 2-methylisoborneol (2-MIB) are associated with the earthy/musty off odors and flavors in municipal water supplies and have been reported as a source of mustiness in wheat grain, apple cider, and cheese, with their production being attributed to actinomycetes, penicillia, and apsergilli. The microorganisms responsible for production of these products in dry navy beans have not been identified.

Purpose: The purpose of this study was to identify the microorganisms associated with the production of geosmin and 2-MIB in raw navy beans.

Methods: Moldy and "good" (control; non-moldy) navy beans were plated in triplicate on actinomycetes isolation agar, potato dextrose agar, dichloran rose bengal agar, and Czapek agar and incubated at 25°C for 5 days. Microorganisms were identified as to genus and species, using traditional methods. Additionally, good navy beans were inoculated (1/10) with "moldy" navy beans (moldy-2), moistened with water, and incubated for 5 days at 25°C; microorganisms were recovered and identified. A gas chromatographic method employing solid phase microextraction was developed to confirm the presence of geosmin and 2-MIB.

Results: *Aspergillus flavus*, *Penicillium expansum*, and *Penicillium commune* were isolated from moldy and moldy-2 beans but not from control beans. Geosmin was not detected in control beans but was detected in moldy and moldy-2 beans at 8–130 ng/kg, with a relative recovery of 12–65%. 2-MIB was detected in control and moldy and mold-2 navy beans at 17–132 ng/kg and 70–100 ng/kg, respectively, with a relative recovery of of 2–48% and 20–70%.

Significance: These data suggest that geosmin is the source of mustiness in raw navy beans. Contamination of navy beans with *A. flavus*, *P. expansum*, and/or *P. commune* is responsible for geosmin production during storage, demonstrating the need for proper storage practices to reduce off flavors in finished bean products.

P5-62 Occurrence of *Carnobacterium* spp. on Retail Samples of Ready-to-Eat Meats

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Lactic acid bacteria (LAB) are associated with a wide variety of meat products, particularly those

stored in the absence of oxygen. The purpose of the investigation was to determine the prevalence of *Carnobacterium* spp. on Ready-to-Eat meat products purchased in the retail marketplace. Samples were purchased during three different sampling periods (3 month intervals). A total of 70 products were selected from different retailers. The product categories (and number of samples tested) included: bologna (8), chicken (10), ham (17), pork (4), roast beef (7), salami (1), turkey (11) and wieners (12). Products were obtained from 15 different establishments in Canada. Products were purchased and stored at 4°C until approximately one week prior to the best-before date. At that time, samples were prepared and plated onto APT, MRS and CTSI (selective for *Carnobacterium* spp.). Plates were incubated anaerobically for 48 or 72 h. A total 547 isolates were selected for further analysis by molecular typing techniques. The first step utilized a multiplex PCR procedure that amplified bands characteristic of both *Leuconostoc* and *Carnobacterium* spp. The 16S-23S intergenic spacer region (ISR) of potential carnobacteria was amplified to identify the 9 different species of *Carnobacterium*. Approximately 25% of the LAB tested were *Carnobacterium* spp. and carnobacteria were detected in all of the product categories tested except the salami. This study illustrates that carnobacteria are an important component of the LAB microflora at the end of the shelf life of vacuum-packaged Ready-to-Eat meat products.

P5-63 DSC Influence of Pressurization Rate and Pressure Pulsing on Inactivation of *Bacillus amyloliquefaciens* Spores during Pressure-assisted Thermal Processing

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Pressure-assisted thermal processing (PATP) is an emerging food processing technology in which a combination of pressure (500–700 MPa) and temperature (90–120°C) is used to inactivate bacterial spores. Systematic studies on the influence of various process parameters on PATP spore inactivation will improve our understanding of the process. The objective of this study was to examine the role of pressurization rate and pressure pulsing in enhancing PATP bacterial spore lethality. *Bacillus amyloliquefaciens* Fad 82 spore suspensions were prepared in deionized water at three inoculum levels (10^9, 10^8, and 10^6 CFU/ml), treated with two pressurization rates (18.1 and 3.7 MPa/s), and held at 600 MPa and 105°C for 0, 0.5, 1, 2, 3, and 5 min. Experiments were carried out with custom-fabricated high pressure microbial kinetic testing equipment. Single and double pulses with the equivalent pressure hold time (1 and 3 min) were investigated by use of the spore suspension containing 10^8 CFU/ml. Spore survivors were enumerated by pour plating, using trypticase soy agar, after incubation at 32°C for 2 days. During short pressure holding times (< 2 min), PATP treatment with the slow pressurization rate provided enhanced spore reduction compared to that of the fast pressurization rate. However, these differences diminished with extended pressure hold times. After 5 min pressure holding time, *B. amyloliquefaciens* population decreased 6.2 log regardless of pressurization rate and inoculum level. Double pulse treatment enhanced spore lethality by approximately 2 to 4 log in comparison to single pulse for a given pressure holding time. Closer examination of the pressure-temperature histories revealed that process temperature during second pulse was about 7–8°C higher during the first pulse. Additional lethal temperature exposure during the second pulse come-up portion might also contribute to enhanced lethality. In conclusion, pressurization rate and multiple pulsing influence the efficacy of PATP treatment.

P5-64 The Survival of *Shigella sonnei* in Frozen Media before and after Ultraviolet Treatment

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Shigella sonnei is a water and foodborne pathogen that can cause dysentery, a type of diarrheic illness. Until recently, edible ice, by definition a food, was not a food safety concern. However, *Shigella* spp. were reported to survive in ice and were epidemiologically linked to diarrheic illnesses. In order to determine the survival of frozen *S. sonnei* F6129 (SF6129), several suspending liquid media were used. An 18 h culture of SF6129 was suspended in the test media and slow and quick frozen at -9°C and -70°C, respectively. On a weekly basis, samples were thawed at room temperature (24 ± 1°C) and plated on either TSA or brain heart infusion agar supplemented with 0.1% Congo red. SF6129 survived for over 3 wks while frozen in 0.85 and 2.0% saline, Butterfield and buffered peptone buffers, vegetable broth and autoclaved water stored either at -9 or -70°C. SF6129 did not survive in filter sterilized tap water which contained residual chlorine, but did survive in the tap water when the residual chlorine was removed by either autoclaving or deionization. When SF6129 was suspended and frozen in Butterfield buffer, ultraviolet treatment (254 nm at 1.2 joules) was effective in reducing the bacterial counts by 3 log. The results of this study showed that SF6129 can survive for reasonable long periods of time (3–6 wks) in ice made from various liquids. UV treatment may provide a decontamination intervention technique that would provide the consumer with pathogen free edible ice.

P5-65 Exploration of Hurdles to Improve Storage Stability of Braised Kidney Beans, a Korean Seasoned Side Dish

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Braised kidney beans are a popular traditional Korean side dish, prepared by heating and mixing ingredients. Systematic investigation of process optimization, packaging and storage conditions has the potential to improve product safety and stability during storage and distribution. Therefore, this study

aimed to find the combinations of preservation hurdles giving maximum storage stability without deterioration of the sensory quality. Formulation adjustment, modified atmosphere packaging and refrigerated storage were examined as potential hurdles to preserve the product. Several quality attributes were monitored during storage at 10°C to determine its primary quality index. Different formulations (concentrations of sugar, corn syrup and soy sauce) and packaging conditions (CO_2 content) were tested with measurement of the primary quality index to find optimal hurdle conditions. Temperature dependence of quality was investigated for the range of 5–20°C. Shelf life of the braised kidney beans was limited by the growth of yeast and mold, and the effect of hurdles on storage stability was quantified by using Baranyi's mathematical model. A formula consisting of 13% sugar, 10% corn syrup and 10% soy sauce based on the boiled beans was the best in inhibiting the microbial growth while maintaining sensory quality. Modified atmosphere of 60% CO_2 and 40% N_2 at 10°C could extend the shelf life by 500% compared to air packaged control. Parameters of microbial spoilage and shelf life estimation function are given for the temperature range of 5–20°C. This study gave a quantified picture of microbial inhibition and shelf-life extension attained by different preservation hurdles. The information may be useful for predicting microbial spoilage of other seasoned side dishes using similar formulations.

P5-66 Inhibitory Effects of Exopolysaccharide (EPS) Produced from *Lactobacillus acidophilus* on the Biofilm Formation of Shiga Toxin-producing *Escherichia coli* O157:H7

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Introduction: Microbial biofilms are complex communities of microorganisms that have been attached to and grown on diverse surfaces. Since biofilms are resistant to antibiotics and are difficult to control in medical and industrial settings, antimicrobial compounds to remove the most resistant biofilms such as mature biofilm should be identified.

Purpose: To determine inhibitory effects of exopolysaccharide (r-EPS) from *Lactobacillus acidophilus* A4 on biofilm formation of Shiga-toxin producing (STEC) *Escherichia coli* O157:H7, a foodborne pathogen.

Methods: STEC O157:H7 was allowed to grow on polystyrene 96-well microtiter plates in 1/2 LB medium containing cell extract, cell bounded-EPS (cb-EPS) and r-EPS, respectively at 30°C for 48 h. Quorum sensing assay was performed with supernatants presumably containing crude autoinducer 2. Biofilm formation was observed using the flow cell chamber (3 x 3 mm) system with a fluorescent microscope.

Results: In the 96-well polystyrene microtiter plates containing 1.0 mg/ml of r-EPS, biofilms significantly decreased by 87%, compared to the plates containing cell extract and cb-EPS. Similar inhibitory effect (95% decrease) was observed on a polyvinyl chloride surface. In these studies, neither growth rate nor AI-2 like activity was affected. In addition, initial attachment and autoaggregation of STEC O157:H7 were inhibited by r-EPS. These results correlate with the fact that biofilm formation was consistently reduced when r-EPS was applied to the continuous-flow chamber mode. These results suggest that r-EPS prevents biofilm formation of a wide range of Gram-negative pathogens.

Significance: r-EPS as a natural biofilm inhibitor can be used in controlling biofilms in food industry in the future.

P5-67 Black Sapote Puree Preserved by Hurdle Technology

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Black sapote (*Diospyros digyna*) is a climacteric, very soft and rapidly perishable fruit grown in Southern Mexico and Central America. Due to its delicate flavor and several unfavorable changes associated with freezing and heat treatments, hurdle technology could be an alternative preservation process for black sapote. The purpose of this work was to develop a stable fresh-like black sapote puree by use of hurdle technology. Ripe sapotes were washed and hand peeled, the pulp was deseeded and the purée adjusted to pH 4.1 by adding citric acid. The puree was divided into three batches, one control; and the other two were adjusted to selected water activities (0.96 or 0.97) by sucrose addition. To the a_w adjusted purees, 100 ppm potassium sorbate were added. Samples were kept at 4°C during 15 days and periodically evaluated for native flora (mesophilic bacteria, yeasts and molds counts), pH, a_w, soluble solids, titrable acidity, instrumental color, and sensory evaluation (5 point hedonic scale). The results were analyzed and compared by ANOVA. Microbial inhibition was observed during storage, and was significantly lower ($P < 0.05$) than for the control. Control sapote spoiled three days before the end of storage. The acidity of the preserved sapote puree with a_w 0.97 was higher than for the puree with a_w 0.96. The sensory panel preferred the preserved sapote puree with higher acidity. Changes in instrumental color were observed during storage with overall sensory acceptability (mean values higher than 3.5) of purees not affected. The pH, a_w, and soluble solids of preserved sapote purees did not change during storage. The hurdle technology preservation process for a fresh-like Sapote puree is effective in both sensory and microbial stability, increasing the shelf life of the product.

P5-68 Effect of Phytosterols on Microbial Spoilage of Pasteurized Fluid Milk

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Introduction: Phytosterols have the ability to reduce serum cholesterol in humans and are now

being added to various foods worldwide, particularly dairy products. Based on recent studies of antioxidant activity, they may also have the potential to impart other benefits often exhibited by phytochemicals, including antimicrobial activity.

Purpose: The purpose of this study was to investigate the antimicrobial effect of several commercial phytosterol mixtures against spoilage bacteria in pasteurized milk.

Methods: A commercial phytosterol preparation (CPP) containing ß-sitosterol, campesterol, sitostanol and campestanol was added at a concentration of 1.8 % (w/v), along with maltodextrin and carrageenan at 0.186 and 0.018% (w/v) respectively, to pasteurized milk stored at 4–7°C. A time course growth study was initiated using appropriate controls and duplicated with milk containing a dispersible phytosterol mixture (mixture of CPP containing maltodextrin, carrageenan and a surfactant agent: sodium stearoyl lactylate (SSL; 0.03%, w/v)) at a concentration of 0.72% (w/v). At predetermined intervals, milk samples were analyzed for standard plate count (SPC), psychrotrophs and *Pseudomonas fluorescens* (added as an inoculum).

Results: CPP exhibited no effect on either SPC or psychrotrophs in milk. CPP and the dispersible mixture of CPP also had no effect on the growth of *P. fluorescens* (perhaps due to higher numbers). However, compared to the controls, the dispersible mixture of CPP with 0.03% (w/v) SSL, when incorporated into milk, inhibited SPC and psychrotroph growth by more than 4 log CFU/ml over 21 days, while SSL without CPP in milk exhibited no inhibitory effect.

Significance: These results indicate that increased dispersibility of phytosterols may increase the shelf life of milk by inhibiting microbial spoilage. Combined with the ability to lower serum cholesterol levels and their antioxidant activity, phytosterols have the potential to be used as multifunctional food ingredients.

P5-69 Effect of High-hydrostatic Pressure on Pathogenicity and Structural Integrity of *Eimeria acervulina*, a Protozoan Parasite and *Cyclospora cayetanensis* Surrogate

ADRIENNE E. H. SHEARER, Gary C. Wilkins, Mark C. Jenkins, and Kalmia E. Kniel, University of Delaware, Dept. of Animal and Food Sciences, 531 S. College Ave., 045 Townsend Hall, Newark, DE 19716, USA

Introduction: High-hydrostatic pressure processing (HPP) has been shown to inactivate various microorganisms and is utilized commercially for enhanced food safety and quality. *Eimeria acervulina* is a protozoan poultry parasite that is closely related to, and has been used as a surrogate for, *Cyclospora cayetanensis*, a human pathogen associated with fresh produce.

Purpose: HPP efficacy against *E. acervulina* was evaluated for insight on potential application of HPP against foodborne human protozoan parasites.

Methods: *E. acervulina* oocysts were treated by HPP at 550 MPa for 2 min at 4, 20, or 40°C in laboratory media and were evaluated for pathogenicity in chickens, their natural host. To help elucidate the mechanism of HPP effects, the structural integrity of HPP-treated *E. acervulina* was evaluated with regard to ease of mechanical breakage, dye permeability, excystation, and microscopy.

Results: Pressure treatment of *E. acervulina* oocysts rendered the parasites nonpathogenic to chickens. Pressure treatment at 40°C also prevented fecal shedding of oocysts. No changes in the fragility and permeability of the oocyst wall or excystation of sporocysts were observed as a result of pressure treatment. Light and scanning electron microscopy revealed no changes to the whole oocyst or sporocysts. Recovery and the morphology of excysted sporozoites were altered by pressure treatment.

Significance: These data illustrate that HPP is an effective method of inactivating *E. acervulina* in laboratory media, and the sporozoite is at least one structure adversely affected by pressure. HPP is worthy of evaluation for inactivation of *E. acervulina* and related protozoan parasites in human foods.

P5-70 Efficacy of Air Cleaning System for Control of Airborne Microbes in Meat Processing Environments

JITU PATEL, Xiangwu Nou, and Gabriel Sanglay, USDA-ARS-BARC-East 10300 Baltimore Ave., Bldg. 201, Beltsville, MD 20705, USA

Introduction: Air is a potential source of microbial contamination in meat processing plants. The airborne contamination of pathogenic and spoilage microorganisms in these operations may impact public health. Intervention strategies to control all sources of contamination of meats, including airborne contaminants, should be included for product safety and quality.

Purpose: The objective of this study was to determine the effectiveness of AirOcare reactive oxygen species (ROS) generating equipment in reducing airborne bacteria in a meat processing environment.

Methods: Equal volumes of *Serratia marcescens* and lactic cultures (*Lactococcus lactis* subsp. *lactis* and *Lactobacillus plantarum* were transferred to a 6-jet Collison nebulizer and meat processing room was aerosolized. Immediately after aerosol generation and at 2, 4, 8 and 24 h intervals, air samples were pulled from each location using Staplex 6 stage air sampler at a flow rate of 28.3 L/min. Aerial populations of lactic acid bacteria and *S. marcescens* were determined by use of MRS and R2A plates, respectively. Surviving bacterial populations were converted to log CFU/m^3 and analyzed by ANOVA.

Results: More than 4 log reductions in *S. marcescens* populations of this bacterium were observed within the first 2 h of treatment ($P < 0.05$) compared to 1 log reduction in the control treatment. The *S. marcescens* populations were reduced by ~4.5 log CFU/m^3 after 24-h exposure to ROS. Approx. 3 log CFU/m^3 reductions in lactic acid bacteria was observed following 2-h exposure with ROS. Further exposure with ROS reduced lactic acid bacteria in air; however, the difference in their survival after 24-h exposure was not significantly different from control treatment.

Significance: These findings reveal that reactive oxygen species treatment using the AirOcare unit significantly reduces airborne *S. marcescens* and lactic acid bacteria in the meat processing environment within 2 h. The treatment is more inhibitory to *S. marcescens* than to the lactic acid bacteria.

P5-71 Selection of Methods for Heat Process Evaluation

JOY E. GAZE and Nick May, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK

Introduction: This project is investigating the application of different methods of evaluation of bacterial destruction in heat processes. Comparisons are made between temperature probes, time-temperature indicators based on enzyme destruction (TTIs) and bacterial spore reduction.

Purpose: To obtain data on the evaluations and assess their applications and resolve any relative strengths and weaknesses in different heat processing regimes.

Methods: Current studies are in the pasteurization area at two ranges, 65–75°C and 85–95°C, with samples held for 1.5 to 30 min. The temperature probes included two type T thermocouples, a needle probe (1 mm diameter) and a twisted pair wire (0.4 mm). A *Bacillus amyloliquefacies* amylase and a *Bacillus licheniformis* amylase TTI were chosen. These were compared to spores of *Clostridium butyricum* and *Bacillus pumilus*.

Results: The data analysis method used so far in the project has been to compare P values calculated from spore and TTI methods against those obtained from thermocouples. Good agreement has been found between mean values under isothermal conditions throughout the temperature range, with maximum discrepancy mean values of +/-P 5 and in most cases less than P 1. Further analysis will include the use of non-isothermal conditions to simulate the come-up time experienced in many food products and an assessment of the variation of D and z values in different substrates.

Significance: These data will confirm the suitability of these methods for verification/validation purposes. Any advantages and disadvantages for particular use will be highlighted and advice given.

P5-72 Isolation and Identification of Radiation Resistance Bacteria from Gamma-irradiated Foods

Jae Hun Kim, P. M. Jeong, J. N. Park, J. K. Park, J. W. LEE, and M. W. Byun, Radiation Application Research Division, Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, 580-185, Korea

Introduction: Although irradiation technique is widely used to sterilize foods, radiation resistance microbes will remain in the irradiated foods. There is a possible problem in achieving the shelf stability and safety of the irradiated foods.

Purpose: This study was conducted to provide information on the radiation resistant microorganisms found in various irradiated foods.

Methods: Cooked bean paste soup and Ready-to-Eat ham sandwiches were subjected to high dose gamma irradiation at 30 kGy for sterilization. Cooked and dried kimchi fried rice and beef fried rice were gamma-irradiated at a middle dose of 10 kGy for pasteurization. Persimmon punch was irradiated with a low dose of 5 kGy. Each of 3 strains isolated from a single colony from irradiated samples using TSA media were then pure cultured. Bacteria 16S rRNA genes were analyzed using two primers, 27°F and 1492R by PCR technique. The amplified 16S rRNA gene was sequenced according to automated sequencing system, and a sequence similarity search was performed with the BLAST Gene Bank data library.

Results: The results distinctly showed active isolates of *Bacillus subtilis* (2 strains) and *Bacillus licheniformis* (1 strain) from kimchi fried rice, whereas the *Bacillus licheniformis* (1 strain) and *Enterococcus gilvus* (2 strains) were observed in beef fried rice and persimmon punch bean-paste soup and ham sandwiches showed the presence of dynamic *Bacillus subtilis* (9 strains).

Significance: Spore-forming *Bacillus* spp. were identified as the highly radiation resistant bacteria throughout the various foods. *Enterococcus gilvus* was identified as a new type of radiation resistant microorganism.

P5-73 DSC Quality and Mold Growth Effects Following Microwave Commercial Sterilization on White Enriched Bread for Military Rations

ALEJANDRO ECHEVERRY, Donna G. Lakins, Christine Z. Alvarado, J. Chance Brooks, and Mindy M. Brashears, Texas Tech University, Dept. of Animal and Food Sciences, Box 42141, Lubbock, TX 79409, USA

Meals Ready-to-Eat (MRE) are a self-contained, flexible package used by military personnel while in the field to store meals for an extended period of time. However, the inclusion of enriched white bread in an MRE posses many problems, especially with regard to shelf-life stability, due to mold growth. A new commercial technology of microwave sterilization was investigated to determine if mold growth was suppressed without detrimental effects on quality over a 60 day period. One loaf of bread was used for quality while the other was inoculated with approximately 1×10^4 CFU/g of a cocktail mixture of 3 separate mold strains obtained from ATCC. At day 0, the bread was exposed to 0, 5, 6, 7, 8, 9 and 10 s microwave (2.45 GHz; 12.2 cm wavelength) treatments. The treated and control bread were stored at 25°C for 60 days and monitored for mold growth, moisture, water activity (a_w), softness, and sensory analysis. There was no quantifiable mold present at day 0 in bread treated for 10 s ($P < 0.05$). By day 60, the bread treated with 10 s treatment had significantly lower counts (> than 3 log) than the bread treated with remaining treatments. Microwave treatment significantly decreased the moisture content of the bread but was not detectable by consumers. There was no difference in (a_w) with microwave treatment though day 45, but differences were detected at day 60 (0.87 and 0.85). There were no differences in softness (mm) of the treated and untreated bread

through day 60. Sensory analysis was conducted using the control and the microwave 10 s treatment, with no difference detected. Microwave commercial sterilization can be used to extend the shelf life of white enriched bread from 1 week to a 2 month period shelf life with minimal mold growth and no detrimental effect on quality.

P5-74 Growth and Recovery of *Lactobacillus fermentum*, *Zygosaccharomyces bailii* and *Aspergillus niger* in a Low pH Juice Beverage in the Presence of Sodium Benzoate

PATRICIA L. RULE, bioMérieux, Inc., 595 Anglum Road, Hazelwood, MO 63042, USA

Sodium benzoate is a commonly used preservative in food and beverages to protect flavor and extend shelf life. It has low toxicity to humans and has very little taste making it a good preservative source. FDA has labeled it as GRAS (Generally Recognized as Safe) and has set allowable levels up to 0.1%. It is even found in "edible" pharmaceutical products such as mouth wash and tooth paste. It has higher antimicrobial activity in lower pH products (pH < 4.5) such as fruit juices, preservatives and salsa products. A retail study of different brands of strawberry banana juice demonstrated anomalies in the recovery of lactics, yeast and mold from the product. although all listed sodium benzoate in the label. *Lactobacillus fermentum* survived and grew well in the similar juice products at a very low inoculum level (3–31 CFU), whereas *Zygosaccharomyces bailii*, and *Aspergillus niger* were completely inhibited or delayed by two days even at higher spoilage levels of 100–300 CFU suggesting that not all lactic acid bacteria and fungal spoilage organisms are created equal in their response to sodium benzoate containing juices. Since retail product does not specify the amount of sodium benzoate present in the product, a separate study is being conducted to review the survival and recovery of *L. fermentum*, *Z. bailii* and *A. niger* at sodium benzoate concentrations of 0.1%, 0.05% and 0.01% at three different pH levels (pH 2, 3 and 4) by continually monitoring the growth via CO_2 production. The continuous monitoring of the CO_2 byproduct of the spoilage organisms can provide incite into the true bacteriostatic nature of sodium benzoate that cannot be observed by end point plating methods that primarily provide bactericidal information.

PROGRAM ADDENDUM

AS OF JULY 2, 2007

MONDAY MORNING — JULY 9

S3 **Food Defense Research and Application**

8:30 a.m. Presenter Change — Food Defense Research Activities at FDA-CFSAN — Faye Feldstein, FDA-CFSAN, Office of Food Defense, Communication, and Emergency Response, College Park, MD, USA

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S4 **Outreach Programs to Promote Dairy Products and Their Safety around the World**
Sponsored by the IAFP Foundation

8:30 a.m. Speaker Change — Reducing Technical Barriers to Dairy Trade — Diane Lewis, U.S. Dairy Export Council, Arlington, VA, USA

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P1 **Dairy, Seafood, Produce and Education Poster Session**

P1-08 Withdrawn

P1-22 Withdrawn

P1–26 Depuration in Cold and Ambient Water Changes the Microbiological Profile of Gulf Coast Oysters – will be presented by D. L. Marshall, Mississippi State University, Starkville, MS, USA

P1-45 Evaluation of the Effect of Lactic Acid Bacterial Isolates on the Growth of *Escherichia coli* O157:H7 and *Salmonella enterica* subsp. *enterica* on Alfalfa Sprouts — will be presented by Divya Jaroni, University of Nebraska-Lincoln, Gretna, NE, USA

P1-59 A Nationwide Study of UK Consumer Attitudes towards Food Safety in the Domestic Kitchen — will be presented by Christopher J. Griffith, University of Wales Institute-Cardiff, Cardiff, Wales, UK

P1-65 Does Recordkeeping Improve Food Safety? — will be presented by Amy Stillings, Eastern Research Group, Inc., Lexington, MA, USA

P1-67 Withdrawn

MONDAY AFTERNOON — JULY 9

S8 **Recent Pivotal Decisions of the National Conference on Interstate Milk Shipments**
Nutcracker 3

1:30 p.m. Presenter Change — The Safest Milk Supply for All the People — Steven Sims, FDA, College Park, MD, USA

2:00 p.m. Presenter Change — International Grade A?... — Dennis Gaalswyk, Quality Chekd Dairies Inc., Naperville, IL, USA

2:30 p.m. Presenter Change — Big Changes for Aseptic Grade A Milk — Allen R. Sayler, International Dairy Foods Association, Washington, D.C., USA

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P2 **Meat and Poultry Poster Session**

P2-03 *Salmonella* Contamination of Cattle between Feedlot and Abattoir — will be presented by Patricia Desmarchelier, Food Science Australia, Brisbane, Queensland, Australia

P2-34 Control of Foodborne Pathogens in Ground Beef Using Controlled Phase Carbon Dioxide — will be presented by Beth Ann Crozier-Dodson, Kansas State University, Manhattan, KS, USA

P2-54 Mechanisms of *Salmonella* Persistence during Chicken Slaughter — will be presented by Patricia Desmarchelier, Food Science Australia, Brisbane, Queensland, Australia

P2-59 Withdrawn

P2-62 Evaluation of Sponge-sampling Methods for Fleece and Carcasses of Sheep in a Commercial Abattoir — will be presented by Patricia Desmarchelier, Food Science Australia, Brisbane, Queensland, Australia

P2-81 Time Change (Was P4-42) — Role of Crude Canola Extracts on *Listeria monocytogenes* Cell Invasion to CaCo-2 Cell Line — will be presented by Vanija Kallur, Alabama A&M University, Normal, AL, USA

TUESDAY MORNING — JULY 10

S10 **The Impact of Emerging Food Trends on Food Safety**
Sponsored by ILSI North America Technical Committee on Food Microbiology

9:00 a.m. Speaker and Title Change — Probiotics, the Different Challenges from an Industrial Perspective — Fabrizio Arigoni, Nestlé Research Center, Lausanne, Switzerland

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Special Interest Session: *Salmonella* Growth, Persistence and Survival in Low-moisture Foods and Their Environments–Strategies for Control

9:00 a.m. Title Change – *Salmonella*: The Organism, Its Habitat and the Role of Testing – ANN MARIE MCNAMARA, Silliker, Inc., South Holland, IL, USA

11:00 a.m. Title Change – The Re-birth of *Salmonella* – DON ZINK, FDA-CFSAN, College Park, MD, USA

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S11 **Food Allergies: A Growing Food Safety Concern**

11:00 a.m. Speaker Change — Food Allergy Management Best Practices — Larry Kohl, Walt Disney World Company, Lake Buena Vista, FL, USA and Paul Marra, Wegmans Food Markets, Inc., Rochester, NY, USA

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P3 **Epidemiology and Risk Assessment, Novel Laboratory Methods, and Applied Laboratory Methods Poster Session**

P3-13 Author List Correction — Estimation of the Burden of Gastroenteric Diseases Study in Miyagi Prefecture, Japan, Using Physician Consultation Rates from a Retrospective Cross-sectional Telephone Survey — KUNIHIRO KUBOTA, Hajime Toyofuku, Fumiko Kasuga, Emiko Iwasaki, Tomomi Nokubo, Shunichi Inagaki, Hei-chiro Kusakari, Mayumi Komatsu, Frederic J. Angulo, Kaoru Morikawa, and Elaine Scallan, National Institute of Health Sciences, Tokyo, Japan

P3-33 Quantification of *Salmonella* from Pig Feces by Real DSC-time PCR — will be presented by Maria Teresa Destro, Cornelia Bunge, and Burkhard Malorny, Federal Institute for Risk Assessment, Berlin, Germany

P3-60 A Comparative Study of Four Alternative Methods and the ISO 6579 Method for the Detection of *Salmonella* spp. in Food Products — will be presented by Jean-Louis Pittet, bioMérieux, R&D Microbiologie Industrielle, Marcy l'Etoile, Lyon, France

P3-61 Withdrawn

P3-62 Withdrawn

P3-77 Withdrawn

P3-78 A Study to Evaluate Direct Plating onto Chromocult® Coliform Agar for the Enumeration of Fecal Coliforms and *Escherichia coli* in Raw Shellfish — will be presented by Bree-Ann Lightfoot, Canadian Food Inspection Agency, Dartmouth, NS, Canada

TUESDAY AFTERNOON — JULY 10

T4 Dairy Technical Session

T4-03 Use of 3M™ Petrifilm™ Aerobic Count Plates for the Detection of *Geobacillus stearothermophilus* in UHT Milk — will be presented by Santos García, Universidad Autónoma de Nuevo León, San Nicolas, NL, Mexico

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P4 Beverages and Water, Antimicrobials, Sanitation and Non-microbial Food Safety Poster Session

P4-13 Antimicrobial Efficacy of Peroxy Foam against Foodborne Pathogens, Yeasts and Molds — will be presented by Mark Carter, Silliker, Inc., South Holland, IL, USA

P4-42 Moved to P2-81

P4-70 Withdrawn

P4-73 Author List Correction — Reduction of the Allergenicity of Hypoallergenic Formula by Gamma Irradiation — JU WOON LEE, J. H. Seo, J. H. Kim, H. J. Kim, B. S. Song, S.Y. Lee, and M.W. Byun, Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

WEDNESDAY MORNING — JULY 11

S18 Preparing Scientists for the Legal Aspects of a Crisis: Step into an Interactive Mock Trial and Learn How to become an Expert Witness

CHANGE OF PROGRAM

8:30 a.m. Donna Garren, National Restaurant Association, Washington, D.C., USA

8:45 a.m. Toni Hofer, Raley's and Bel Air, West Sacramento, CA, USA

9:00 a.m. Melvin Kramer, Epihealth Associates, Baltimore, MD, USA

9:15 a.m. David A. Ernest, Bullivant, Houser, Bailey, Attorneys at Law, Portland, OR, USA

9:30 a.m. Chris Domenic, Tsongas Litigation Consulting, Portland, OR, USA

10:00 a.m. Break

10:30 a.m. Mock Trial and Summary

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S20 Food Safety @ the Speed of Thought – Creating Virtual Networks

Meet Your Panel of Experts

Collaboration with Global Teams and Use of Emerging Technologies — Frank Farro, Disney Parks & Resorts, Lake Buena Vista, FL, USA

Hands-on Demonstration of Technology Tools Used for Collaboration: How and Why You Should Use These Tools — WebEx Communications, Inc.

Human Factors to Consider When Using Collaboration Tools — Allen Fazio, Disneyland Resort, Anaheim, CA, USA

Panel of Information Technology Experts

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P5 Food Defense, Pathogens and General Microbial Poster Session

P5-40 Quantitative Evaluation of Human Norovirus Persistence in Different Types of Water — will be presented by Julie Jean, Universite Laval, Quebec, QC, Canada

P5-54 Carriage of *Escherichia coli* O157 by European Starlings – will be presented by Jeffrey LeJeune, The Ohio State University, Wooster, OH, USA

P5-57 Microbiological Evaluation of Infant Bottles Used for Feeding Powdered Formula: Implications for *Enterobacter sakazakii* — will be presented by Christopher J. Griffith and Steven Riley, University of Wales Institute-Cardiff, Cardiff, Wales, UK

P5-72 Author List Correction — Isolation and Identification of Radiation Resistance Bacteria from Gamma-irradiated Foods — Jae Hun Kim, P. M. Jeong, J. N. Park, J. K. Park, J. K. Kim, J. W. Lee and M.W. Byun, Korea Atomic Energy Research Institute, Jeongeup, Korea

WEDNESDAY AFTERNOON — JULY 11

RT6 Food Safety Laws: Political Science or Food Science

Sponsored by the IAFP Foundation

ORDER OF PRESENTATIONS HAS CHANGED

1:30 p.m. Viewpoint: National Academy of Sciences, Academic — Michael P. Doyle, Center for Food Safety, University of Georgia, Griffin, GA, USA

2:00 p.m. Viewpoint: Former US Food Safety Regulator — Mike Taylor, University of Maryland, School of Public Health, Baltimore, MD, USA

2:30 p.m. Viewpoint: International, World Health Organization — Peter Ben Embarek, World Health Organization, Geneva, Switzerland

3:00 p.m. Roundtable Discussion— Frank Yiannas, Walt Disney World Company, Lake Buena Vista, FL, USA–Moderator
Questioner: Jenny Scott, GMA/FPA, Washington, D.C., USA