Technical Abstracts

T1-01 Aspects of Systems Theory in the Analysis of Molecular-biological Based Detection Methods

PETER ROSSMANITH and Martin Wagner
University of Veterinary Medicine Vienna, Christian Doppler Laboratory for Molecular Food Analytics, Vienna, Austria

Introduction: The implementation of molecular-biological based food-pathogen detection is a frequently and intensively discussed topic. Molecular-biological methods for food analysis comprise a detection chain consisting of sample preparation, target purification and a detection assay. Given this systemic character systems theory provides basis for discussion of principles and application of testing methods derived from various scientific areas for specification and validation of pathogen detection.

Purpose: This work describes the structure and strategy of a possible alternative approach for validation and specification of molecular-biological methods to accelerate their broad range implementation into food pathogen detection.

Methods: The hypothesis is established that systems theory provides the basis for implementation of test systems, derived from other scientific or technical areas, to specification and validation of molecular-biological food pathogen detection methods, or alternatively as supplemental to existing international standards. The categorization of black box and white box systems demonstrates a possible classification for pathogen detection methods. Furthermore, the transformation of models from systems theory to the analytical chain of food detection provides new insights for opportunities and restraints for such methods, especially when compared with conventional microbiological methods.

Results: The resulting applicability of Physical-Modeling-Synthesis and System-Identification as used in systems analysis provides two strong instruments for validation of the completely molecular-biological detection process. Equivalence-Class-Formation and Limit-Analysis, which is the underlying test principle for approvable application of System-Identification by means of Poisson-Analysis, both support specification of the enzymatic assay building the core of a molecular-biological detection chain. This alternative approach is based on validation of the method per se and supports conventional comparative validation according to ISO 16410.

Significance: The application of systems theory to problems of food detection by molecular-biological methods provides a strong tool and an alternative approach for the validation of new methods, specification of the enzymatic core method and evaluation of related and unanswered questions in this context.

T1-02 Utilization of Evolutionary Model, Bioinformatics and Heuristics for Development of a Multiplex *Escherichia coli* O157:H7 PCR Assay

FRANK R. BURNS and Jim Bono
DuPont, Wilmington, DE, USA

Introduction: E. coli O157:H7 is a devastating foodborne pathogen causing many foodborne outbreaks world wide with significant morbidity and mortality. The plasticity of the *E. coli* O157:H7 genome, the inconsistent expression of surface antigens, and the sharing of genetic elements with other non-pathogenic bacteria (many of whom have yet to be characterized) complicates the development of a reliable and specific PCR assays for this organism.

Purpose: The purpose was to develop a PCR based assay for *E. coli* O157:H7 that would be inclusive of all *E. coli* O157:H7 isolates, including all known atypical isolates (e.g., rough, sorbitol-fermenting, telluride-sensitive isolates), while maintaining sufficient specificity for use as a reliable screening tool.

Methods: The genetic evolutionary model for *E. coli* O157:H7 was used to determine, broadly that specific targets from both the O55:H7 parental lineage as well as the O157 encoding gene cluster (acquired as the last step in the evolution of this pathogen) should be employed. Bioinformatics and heuristic approaches were undertaken to identify multiple candidates from both these lineage elements. Targets were then tested against over 250 *E. coli* O157:H7 isolates from both the DuPont and USDA Meat Animal Research Center strain collections. In addition these targets were tested against 350 ground beef enrichments to evaluate potential for non-specific cross reaction with genetically uncharacterized bacteria.

Results: Of the eight targets evaluated, two failed at being completely inclusive; both missed isolates of the sorbitol-fermenting, telluride-sensitive lineage responsible for several European outbreaks. Of the remaining six targets, three had acceptably low rates of non specific cross reactivity, two of these from the O157 gene cluster. The first target chosen has greater specificity utilizing two single nucleotide polymorphisms and is non-reactive with most O157-bearing bacteria that are not O157:H7. The second target chosen was from the O55:H7 parental strain, and exploits de novo nucleotides flanking a truncated and disabled insertion sequence.

Significance: An understanding of *E. coli* O157:H7 evolution, coupled with bioinformatics and heuristic approaches to dealing with largely uncharacterized background flora has allowed the development of a highly specific and inclusive multiplex PCR assay without the complications and failure modes introduced by upfront antibody based selection.

T1-03 A Novel Colorimetric Screening Assay for *Escherichia coli* O157:H7 In Raw Ground Beef and Trim Utilizing Simultaneous Capture and *In Situ* Labeling during Automated Re-circulating IMS

Nicole Prentice, John Murray, Paul M. Benton, Katarzyna Brzegowa, Brooke V. Houston, Ian Sheldrake, Michael F. Scott, Christine Aleski and ADRIAN PARTON
MATRIX MicroScience Ltd. Cambridgeshire, United Kingdom

Introduction: E. coli O157:H7 is notorious because of its low infective dose and the severity of the disease in vulnerable individuals. Raw ground beef has been implicated as the source of *E. coli* O157:H7 in a significant number of food-borne disease outbreaks and food safety recalls during the past two decades. Detecting the presence of this STEC at low levels in raw beef presents significant challenges to both the food industry and regulatory agencies.

Purpose: This study describes the development and validation of a robust screening assay for *E. coli* O157:H7 in raw fresh beef samples based on *in situ* labeling of the target STEC captured during re-circulating IMS. The method is applicable to analyzing both ground beef and trim samples for the presence of *E. coli* O157:H7 where initial pathogen levels are in the 1—5 CFU per sample range.

Technical

Methods: Fresh ground beef patties or trim (25—375 g) were weighed into sterile stomacher bags; each sample received a low level inoculum (1—5 CFU) of cold stressed nutrient starved *E. coli* O157:H7 to mimic very low-level contamination. Samples were diluted 1:9 with pre-warmed Buffered Peptone Water and samples were briefly hand mixed prior to static pre-enrichment (7—16 h) at 42°C. Pathatrix re-circulating IMS was used to capture *E. coli* O157 from pre-enrichment aliquots and simultaneous specific labeling of target was achieved with an immunoconjugate having the same specificity as the capture ligand. Following automated wash and elution steps, Pathatrix beads were immersed in an enzyme substrate. Development of a blue color was indicative of the presence of *E. coli* O157:H7 in the sample. A proportion of the recovered Pathatrix beads were also streak plated onto selective agar plates for confirmation.

Results: Reliable detection of initial low level *E. coli* O157:H7 in raw ground beef and trim samples was achieved using the labeling of target cells in conjunction with the automated RIMS capture and washing procedure. No natural *E. coli* O157:H7 in uninoculated raw beef samples was encountered. Based on comparing the screening assay result with target isolation on selective agar plates no false positive or false negative results were obtained. Recovery of *E. coli* O157:H7 colonies on selective agar plates confirmed the colorimetric screening assay result in all cases.

Significance: The *E. coli* O157:H7 screening method described in this study offers a flexible and cost effective approach to identifying the presence of this STEC in beef samples. It offers next day results and reliability. User handling is minimized as target capture, labeling and washing steps are an integral part of the automated RIMS procedure.

T1-04 Sensitive and Rapid Detection of *Escherichia coli* O157:H7 in Food and Water

PEIXUAN ZHU, Shuhong Li, Platte T. Amstutz, Daniel R. Shelton and Cha-Mei Tang
Creatv MicroTech, Inc., Potomac, MD, USA

Introduction: FDA and USDA follow a zero tolerance policy for *Escherichia coli* O157:H7 contamination in food. Sensitive and rapid methods are needed to determine the presence of *E. coli* O157:H7 in food and water.

Purpose: An assay was developed to detect *E. coli* O157:H7 in food and water at low concentrations. The short enrichment time enabled rapid results.

Methods: The assay is based on Immunomagnetic Separation- Fluorescence Immunoassay (IMS-FIA). Antibody-coated magnetic beads were added to test samples to specifically capture *E. coli* O157:H7 cells. Bead-bound cells were recognized by an anti-O157:H7 Cy5-labeled polyclonal antibody to form an immuno-sandwich. Unbound dye was removed by washing. The fluorescence signal from the immuno-sandwich was compared to the fluorescence of a modified assay, where the dye-labeled antibody was dissociated from the magnetic beads. The fluorescence signals were measured using an ultra sensitive Signalyte™-II spectrofluorometer. Ten-fold serial dilutions of *E. coli* O157:H7 were tested.

Results: The results showed that the fluorescence intensity from the supernatant of the modified assay was highly correlated with the original *E. coli* O157:H7 cell concentrations. Limit of detection (LOD) threshold was established based on the average fluorescence intensity, plus three times the standard deviation of the negative controls. Fluorescence signals of the immuno-sandwich assay were more variable and less sensitive than the modified assay. LOD for *E. coli* O157:H7 of the modified assay was 10 CFU/ml in water, over two log better than the original assay. Comparable assays conducted for *E. coli* O157:H7 in ground beef were a factor of 10 less sensitive. Results were consistently reproduced monthly using the same reagents, which demonstrated a shelf life in excess of six months.

Significance: *E. coli* O157:H7 can be rapidly and consistently detected at low concentrations in food and water using the Signalyte™-II spectrofluorometer.

T1-05 Identification of Shiga Toxin-producing *Escherichia coli* on DNA Microarrays by Using a Novel Photoinduced Signal Amplification Method

BEATRIZ QUIÑONES, Ronald P. Haff, Amber W. Taylor and Erica D. Dawson
USDA-ARS, Western Regional Research Center, Produce Safety and Microbiology Research Unit, Albany, CA, USA

Introduction: Shiga toxin-producing *Escherichia coli*, with *E. coli* O157:H7 as a common serotype, is a leading cause of human gastrointestinal illnesses. The rise in foodborne-related outbreaks of pathogenic *E. coli* from food and waterborne sources has heightened the importance of developing improved methods to rapidly detect and characterize virulent strains. Therefore, the development of effective detection methods that identify the presence and distribution of *E. coli* O157 are needed with sufficient sensitivity, cost-effectiveness and suitability for routine testing.

Purpose: To evaluate better methods to rapidly detect and genotype *E. coli* O157 virulent strains, the present study explored the use of photopolymerization, a colorimetric and photoinduced signal amplification detection method, for pathogen identification on DNA microarrays.

Methods: A DNA oligonucleotide microarray was constructed with 30-mer oligonucleotide probes targeting intimin adherence protein (eae), perosamine synthetase (per), Shiga toxin 1 (*stx1*), and Shiga toxin 2 (*stx2*) in pathogenic *E. coli*. For detection on the microarray of these pathogen genetic markers, the microarray was hybridized with biotin-labeled PCR products and then labeled with a streptavidin-conjugated photoinitiator and a solution containing monomers. After irradiating at a specific wavelength, the formation of a colorless, cross-linked hydrogel polymer allowed the colorimetric detection of positive signals on the microarray.

Results: Analysis of the microarray data demonstrated polymer formation for only probes targeting virulence genes present in the tested *E. coli* O157 reference strains. Positive hybridization signals had average signal-to-noise ratio values above 10, and signal-to-noise ratio values below 1.5 were determined for the same virulence probes in a non-pathogenic *E. coli* strain. The quantification analysis of positive signals demonstrated that the detection sensitivity by using photopolymerization was approximately 100—1000 CFU/ml.

Significance: The use of DNA microarrays in combination with photopolymerization allowed the rapid and cost-effective identification of *E. coli* O157, compared to established methods that are more expensive and require several days for strain detection.

T1-06 Rapid Identification of *Listeria* Species: Comparison of a Real-time PCR Assay Versus Biochemical Galleries

Nicolas Desroche, Jean Guzzo and PATRICE ARBAULT
BioAdvantage Consulting, Food Safety and Analytical Methods, Orliénas, France

Introduction: Conventional methods for the detection and enumeration of *Listeria* sp. require biochemical tests to differentiate amongst the 6 species of *Listeria*. These tests are performed from colonies and rely on catalase activity, hemolytic characteristic and sugar fermentation. Rapid biochemical kits such as API® *Listeria* and Microbact™ *Listeria* are available for carrying out these biochemical test panels. Recently, a new automated real-time PCR method has been developed for the rapid identification of *Listeria* species (GeneDisc *Listeria* ID).

Purpose: The objective of this study was to evaluate the performances of this PCR assay and compare them to commercial biochemical galleries: API® *Listeria* and Microbact™ *Listeria*.

Methods: One hundred sixty-six (166) collection strains of the 6 main *Listeria* species were isolated onto agar plates. Colonies were picked up from the plates and then identified with the 2 biochemical galleries and the PCR-based assay, following the respective kit instructions. The *Listeria* identification results were compared. Any strain giving discordant results among the 3 assays was further analyzed through 16S DNA sequencing.

Results: The GeneDisc *Listeria* ID identified correctly all *Listeria* strains (166/166) while both biochemical galleries showed some erroneous results (5/166). The most important error was observed with the misidentification of 3 out of 11 *L. ivanovii* strains. No misidentification was observed for the species *monocytogenes* and *innocua*, using the biochemical galleries. Furthermore, the time to result was significantly lower with the GeneDisc ID *Listeria* (less than 1 hour) compared to the biochemical galleries (approximately 24 hours).

Significance: The automated PCR-based identification method seems a promising tool for the routine and rapid identification of *Listeria* isolates in food and clinical laboratories.

T1-07 Detection of *Listeria* spp. from Pooled Environmental Swab and Food Samples within 24 Hours Using Pathatrix Automated Re-circulating IMS Linked to Real-time PCR

John Murray, Nicole Prentice, Katarzyna Brzegowa, Paul M. Benton, Brooke V. Houston, Ian Sheldrake, Michael F. Scott, Marcie Van Wart and ADRIAN PARTON
MATRIX MicroScience Inc., Golden, CO, USA

Introduction: Listeriosis is an important public health problem that produces high mortality rates. Serious infections occur mainly in pregnant women, neonates, immuno-compromised and elderly individuals and result primarily from eating food contaminated with the bacterium *Listeria monocytogenes*. *L. monocytogenes* has a ubiquitous distribution and possesses properties that increase the risk of its persistence and dissemination in food processing facilities. Like other members of the genus, *L. monocytogenes* is psychrophilic and can grow at refrigeration temperatures of 1°C. The potential implications of *L. monocytogenes* being present in ready-to-eat foods which support growth has led to a zero tolerance approach from both the FDA and USDA-FSIS. RTE Food considered to be high risk for *Listeria monocytogenes* include those with pH > 4.4 and a_w > 0.92.

Purpose: The aim of this study was to assess the feasibility of using an enhanced enrichment protocol, recirculating IMS and real time PCR to develop a method which is capable of detecting the presence of *Listeria* contamination, including *L. monocytogenes*, at low level in pooled food and environmental swab samples within 24 hours.

Methods: A range of foods and environmental contact swabs were inoculated at low level (1-10 CFU per sample) with single *Listeria* species from a representative panel spanning the *Listeria* genus and including *L. monocytogenes*. All samples were diluted in pre-warmed demi Fraser and enriched statically at 37°C for 23 hours prior to pooling aliquots from inoculated samples with uninoculated samples in the ratio 1:4 or 1:9. Pooled samples were analyzed using Pathatrix Auto. Target capture employed IMS particles with proven inclusivity for all *Listeria* species. A 5-minute mechanical lysis step was used to release DNA from captured target cells prior to real time PCR detection. Selective agar plating was used to confirm the PCR results.

Results: The range of *Listeria* spp. were successfully isolated from pooled food samples down to 0.004 CFU/g and environmental contact swabs (1 CFU in 100 cm^2) using recirculating IMS. Detection was achieved using real time PCR and this was confirmed by isolation of target *Listeria* on selective agar plating.

Significance: The Pathatrix *Listeria* pooling method described allows food production facilities to increase sample throughput during routine *Listeria* monitoring of both food and environmental contact swabs. The method has the potential to enhance HACCP and pathogen testing regimes and can be employed to validate hygiene practices and sanitizing procedures aimed at reducing the incidence and spread of *Listeriae* in the food processing environment.

T1-08 Combined Thin Agar Layer and Centrifugation-plating Method for Enumeration of Injured *Salmonella*

Sangha Han and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Detection of sub-lethally injured *Salmonella* in foods is important because the organism may repair and ultimately cause illness. The thin agar layer (TAL) technique applies a non-selective agar to a traditional selective plating medium, which allows for the resuscitation and enumeration of injured cells. The combination of TAL with a centrifugation-plating technique (CP), potentially with a sample pre-treatment step, could allow for the enumeration of both vegetative and injured cells from food matrices.

Purpose: The aim of this study was to examine the efficacy of a combined TAL-CP method for the enumeration of acid and sodium chloride-injured *Salmonella*.

Methods: Strains of five *Salmonella* serotypes were injured by incubation at 5°C for 10 days in a nutrient broth containing NaCl (13.5%) and lactic acid (1%). Following injury, each strain was inoculated into chicken rinse, which was then centrifuged after treatment with protease A and Tween 80. The resuspended cells were enumerated onto tryptone soy agar (TSA), xylose lysine desoxycholate (XLD) and TAL (14 ml of TSA on 14ml of XLD). Counts on each medium were compared to determine recovery rates of injured *Salmonella*.

Results: Sublethally injured *Salmonella* cells were recovered well using CP. There was no statistically significant difference ($P > 0.05$) between TSA and TAL for enumeration of injured *Salmonella*. Both these media recovered higher numbers of injured *Salmonella* than XLD ($P < 0.05$). All three media showed no difference ($P < 0.05$) when inoculated with uninjured cells. Recovery of sub-lethally injured cells of four serotypes was about 90% after treatment with Tween 80 and protease, though recovery of *Salmonella* Infantis was only about 78%.

Significance: This study suggests that the TAL-CP method is effective for enumeration of *Salmonella*, including injured cells.

T1-09 Comparison of Vegetable and Animal Peptone-based Culture Media for Detection of *Salmonella* in Poultry

Rakesh Koorapati and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Peptones, polypeptides obtained from the hydrolysis of proteins, are primary protein sources in culture media; traditionally they are derived from animal sources. However, due to religious beliefs and concerns over transmissible spongiform encephalopathies (TSE), especially the bovine form (BSE), vegetable peptones have become an increasingly important alternative.

Purpose: The aim of this study was to compare the performance between vegetable peptone VP-based and conventional meat peptone (MP)-based culture media, using *Salmonella* as the analyte and poultry products as food matrices.

Methods: Growth of three strains of *Salmonella*, in fully vegetative or injured state, was determined in Buffered Peptone Water (BPW), Rappaport-Vassiliadis Soy (RVS) broth and Muller Kauffmann Tetrathionate novobiocin (MKTTn), as well as on a range of plating media,

Technical

from two commercial manufacturers, one supplying VP media and the other, a market-leader, supplying MP media. Naturally and artificially inoculated chicken samples (n = 80) were screened for the presence of *Salmonella* spp. using an ecometric streaking technique, in order to compare the performance between VP- and MP-based culture media. Plating media were formulated from individual ingredients with peptone as a solitary variable ingredient to assess specifically the performance of the peptones.

Results: No significant differences were observed in the growth of the three *Salmonella* serovars in broth or on solid culture media ($P < 0.05$). Absolute growth indices (AGIs) calculated from ecometric streaking on VP and MP plating media — XLD, Hektoen Enteric, Bismuth Sulphite and Brilliant Green agars — showed no significant difference in productivity rates for *Salmonella* and specificity, in terms of growth of the background microflora. A < 0.7 log CFU/mL difference in recovery rates was observed on media formulated with the same ingredients except VP or MP.

Significance: VP-based media have high specificity, productivity and perform similarly to MP media. Since vegetable peptone media are free from religious and BSE concerns, they serve as suitable replacements for animal peptone-based culture media.

T1-10 Development of Multi-parametric Tools for the Detection and Identification of Sporeforming Bacteria in the Food Chain

PATRICE CHABLAIN, Florence Postollec, Stephane Bonilla, Daniele Sohier and Sylvie Hallier-Soulier
Pall GeneSystems, R&D, Bruz, France

Introduction: Aerobic and anaerobic Gram positive sporeformers show a wide range of phenotypic and genotypic characteristics. These organisms are ubiquitous in the environment and have the ability to form endospores which enable them to survive treatments commonly used in food processes. User-friendly tools were developed for the rapid detection and identification of major sporeformers implicated in food spoilage and food poisoning outbreaks.

Purpose: A multi-parametric PCR-based method has been developed for the detection and identification of the most prevalent genera and species of sporeformers. The method performances were evaluated with both artificially and naturally contaminated samples.

Methods: A 28-h enrichment protocol has been defined to allow germination and growth of aerobic and anaerobic targeted strains. Chelex based extraction and developed GeneDiscs plates allow, at once, multiplex detection of several sporeformers commonly found in food industries. Inclusivity and exclusivity study has been tested on more than 220 collected strains, mostly composed of food isolates.

Results: Specificity study showed few discrepancy due to the taxonomic evolution. The developed biochip yields detection limit lower than 1 spore *B. cereus*/gram while standard detection method on agar yields more than 8 spore/g. PCR performances have been validated with both artificially (n = 60) and naturally contaminated food according to ISO 16140. Sporeformers' vegetative cells and spores present in naturally contaminated samples have been isolated without and with heat treatment, colonies have been 16SrRNA sequenced and identified according to the actual taxonomy in order to confirm the biochip molecular response. *Bacillus* genus was systematically confirmed while *Clostridium* detection could not always been confirmed on standard RCM medium due to difficult recovery on agar medium.

Significance: The lack of diagnosis tools is the major obstacle to control sporeformers contamination. The development of ready-to-use multiple parametric tools enable detection, identification and traceability of 5 genera and 9 species in less than 24 h rather than quantification of a few species. Sporeformers biodiversity and prevalence observed in raw materials or ingredients was not systematically correlated to the observed spoilage of the final product. Nevertheless, molecular detection offers the advantage to track mesophilic or thermophilic anaerobes which are extremely difficult to study with conventional methods.

T1-11 Differentiation and Speciation of Vibrios by PCR of 16S-23S rRNA Intergenic Spacer Region

MARIA HOFFMANN, Eric W. Brown, Peter C. Feng, Markus Fischer and Steven R. Monday
US Food and Drug Administration, CFSAN, College Park, MD, USA

Introduction: The genus *Vibrio* is comprised of 74 species. Among vibrios, *V. parahaemolyticus* and *V. vulnificus* have a greater potential to establish infection in humans, generally following consumption of raw or undercooked seafood. *Vibrio* infections are becoming more common worldwide. The United States Centers for Disease Control and Prevention (CDC) estimates that 8,028 *Vibrio* infections and 57 deaths occur annually in the United States. Of these infections, 5,218 are foodborne in origin. Consequently, it is important to have a method that can reliably identify *Vibrio* isolates.

Purpose: The purpose of this study was to design a PCR-based method using 16S-23S rRNA Intergenic Spacer Region (ISR) to reliably and efficiently differentiate numerous *Vibrio* species.

Methods: 16S rRNA gene sequencing was accomplished for all strains used in this study. *Vibrio*-specific PCR primers were designed to amplify 16S-23S rRNA ISR targets. ISR amplicons were resolved by capillary gel electrophoresis. The resulting patterns were analyzed using BioNumerics fingerprinting software.

Results: The data showed that this method easily discriminated the 69 *Vibrio* type strains at the species level. Furthermore, testing of 36 strains each of *V. parahaemolyticus* and *V. vulnificus*, isolated from numerous geographical locations demonstrated distinct intra-species ISR-typing patterns, making this technique equally useful for intraspecies differentiation, as well. Finally, vibrios isolated from sponges were characterized using16S rRNA gene sequencing and ISR-typing. The resulting data suggested both a panmictic population structure among geographically separate vibrios and evidence that sponge hosts may also be a source for speciation.

Significance: This fast, reliable and efficient ISR-typing system, which takes advantage of capillary electrophoresis technology, has been proven to be effective for identification of *Vibrio* species at both the species and subspecies level, suggesting it should also be useful for epidemiological investigations, as well.

T1-12 Detection of Low Numbers of Only Viable *Enterobacteriaceae* in Inoculated Pasteurized Milk Using Direct PCR after Ethidium Bromide Monoazide Treatment

FRANK SCHLITT-DITTRICH, Takashi Soejima, Tomoko Yaeshima and Keji Iwatsuki
Morinaga Milk Industry Co., Ltd., Biological Function Research Dept., Kanagawa-Pref., Zama-City, Japan

Introduction: The microbial analysis of milk by culturing methods is time consuming and laborious. Application of PCR in milk examination can significantly reduce the analysis time but various milk components inhibit the DNA-amplification. Furthermore, PCR does not distinguish between viable and dead bacterial cells.

Purpose: The purpose of this study was to develop a direct PCR (DPCR) assay that enabled the detection of only viable *Enterobacteriaceae* in milk after Ethidium Bromide Monoazide (EMA) treatment without DNA-isolation and purification.

Methods: Aliquots of 22 ml of pasteurized milk were single-strain inoculated with up to 7-log CFU of viable and heat-killed bacteria, respectively. Prior to DPCR (targeting an approximately 2490 bp DNA-fragment of the 16S to 23S rRNA gene) the sample preparation

and treatment were conducted including the following steps: pelletization, re-suspension and incubation in Brain Heart Infusion broth supplemented with proteinase for 3 h at 37°C, exposure to EMA and visible light, washing, and appropriate dilution in sterile water. The amplified DNA-fragment was identified by its melting point and visualized after gel electrophoresis and SYBRGold staining.

Results: Viable cells of 13 *Enterobacteriaceae* strains (members of 13 different genera) were detected in milk at inoculation levels of 14 ± 8 CFU (n = 26) in duplicates within 8 h of analysis time. Samples initially inoculated with up to 7 log CFU of dead *Cronobacter muytjensii* ATCC 51329 (formerly *Enterobacter sakazakii*) cells and viable Gram-positive bacteria cells (*Bacillus cereus* and *Staphylococcus aureus*) were tested negative with EMA-DPCR.

Significance: The analysis time was reduced to < 8 h which was significantly shorter compared with culturing methods which can take p to several days. We could detect < 10 viable *Enterobacteriaceae* cells in 10 ml of milk.

T2-01 Variation in Desiccation Tolerance among *Salmonella* Strains

REGINA C. WHITEMARSH, Aaron M. Gnas and Charles W. Kaspar
University of Wisconsin-Madison, Bacteriology, Madison, WI, USA

Introduction: *Salmonella* outbreaks associated with low-moisture foods, like peanut butter, powdered milk, and almonds have raised questions about the survival of this pathogen in ingredients and products that have traditionally been thought to inhibit growth and survival.

Purpose: A greater understanding of *Salmonella* persistence in and response to desiccation conditions is critical to developing and implementing intervention strategies.

Methods: Four strains of *Salmonella enterica* were used in this study; two strains (M-09-0001A, peanut butter outbreak isolate and LT2) of serovar Typhimurium, and one strain of serovars Tennessee and Agona. The ability of the strains to survive at low a_w (a_w=0.92) was examined using NaCl and sucrose to adjust the water activity. The influence of storage temperature (15 and 30°C) was also investigated. Survival was monitored by testing samples at time zero and weekly thereafter for the number of CFU/ml using trypticase soy agar.

Results: Survival of the *Salmonella* strains differed in low-a_w solutions prepared with sucrose and NaCl with greater survival in the NaCl-prepared solutions. In general, Typhimurium strain LT2 had the poorest survival. Incubation at 15°C significantly increased the survival of all six strains compared to survival at 30°C with less than a 1-log decrease in CFU/ml after 21 days of incubation at 15°C. *Salmonella* serovars and strains did not differ significantly in survival at 30°C with the exception of strain LT2 and the Enteritidis strain E40. Heat shock of *Salmonella* for an hour at 45°C prior to inoculation of low-a_w solutions enhanced survival.

Significance: These findings are significant because they demonstrate significant survival of *Salmonella* during desiccation. Low temperature or prior exposure to stress (heat shock) enhanced survival in low-a_w conditions. This information should be helpful in the development of effective intervention practices.

T2-02 Effect of Crust Freezing on the Survival of *Escherichia coli* and *Salmonella* Typhimurium in Raw Poultry Products

BYRON D. CHAVES-ELIZONDO, Inyee Y. Han and Paul L. Dawson
Clemson University, Food Science and Human Nutrition, Clemson, SC, USA

Introduction: *Escherichia coli* and *Salmonella* spp. are ubiquitous to the poultry production environment and hence their transmission to poultry products is a concern. Industry has widely used freezing as a strategy to halt pathogen growth and more recently, crust freezing has been claimed to improve operations, quality, and even safety of poultry products.

Purpose: To determine the effect of crust freezing and the presence of skin on the survival of *E. coli* and *S.* Typhimurium in raw poultry products.

Methods: Ampicillin-resistant *E. coli* JM 109 and nalidixic acid-resistant *S.* Typhimurium were used in the experiments. A set of cultures was subjected to cold-shock stress by storage at 4°C for 10 days. Commercial chicken breasts without skin and chicken thighs with skin were inoculated with each bacterium in separate experiments being either cold-shocked or non-cold-shocked prior to inoculation. Samples were crust frozen at -85°C for 20 min or completely frozen at -85°C for 60 min. *E. coli* and *S.* Typhimurium were recovered on appropriate selective and non selective media containing the corresponding antibiotic. Log reductions and injury extent were calculated and treatments were compared using ANOVA.

Results: No significant differences were observed in the reduction of cold-shocked or non-cold-shocked bacteria on products that were crust- or completely frozen, with or without skin. Reductions tended to be greater for *S.* Typhimurium than for *E. coli*, although none of the final reductions were greater than the desired target (1 log). Bacterial cell injury was not significantly different among any of the treatments.

Significance: The treatments did not show practical significance for initial reduction of these pathogens; thus freezing nor crust freezing should not be considered strategies for the reduction of these pathogens on poultry. However, additional studies are underway to compare crust freezing to refrigeration for inhibition of bacteria on raw poultry products.

T2-03 Characterization of the *Listeria monocytogenes* Transcriptional Response to Synergistic Growth Inhibition by Potassium Lactate and Sodium Diacetate

MATT STASIEWICZ, Teresa M. Bergholz and Martin Wiedmann
Cornell University, Food Science, Ithaca, NY, USA

Introduction: Combinations of organic acids are frequently used in the Ready-to-Eat meat industry to slow the growth of *Listeria monocytogenes*, a psychrotolerant foodborne pathogen, in refrigerated products. Although it has been demonstrated that the combination of diacetate and lactate causes greater than additive, i.e., synergistic, growth inhibition, the mechanisms of observed synergistic inhibition as well as how *Listeria* overcomes the inhibitory stress are not well understood.

Purpose: To use transcriptional profiling to investigate the mechanistic basis for diacetate and lactate synergy and identify genetic pathways *L. monocytogenes* uses to overcome their growth inhibitory effects.

Methods: *L. monocytogenes* h7858 and f6854 were exposed to the growth inhibitor treatments (0.14% water phase [w.p.] sodium diacetate, 2% w.p. potassium lactate, the combination of 0.14% diacetate and 2% lactate, or no inhibitors as control) in broth at 7°C for 8 h, and RNA was extracted and competitively hybridized to microarrays. ANOVA comparing the four treatments for each strain was used to identify differentially expressed genes (P < 0.05, fold-change > 1.5).

Results: The transcriptional response included 593 and 282 genes for h7858 and f6854, respectively, with a significant interaction between lactate and diacetate treatments; of these genes, 90 were significant in both strains. Cluster analysis showed the majority of the significant genes had either increased or decreased expression in both diacetate and lactate treatments compared to control and those

Technical

differences were magnified in the combination treatment. Gene Set Enrichment Analysis found some responses shared between strains, e.g., strong induction of fermentation genes particularly oxidoreductases and alcohol dehydrogenases, yet only 3 of 14 total role categories enriched were shared, suggesting the strains have very different transcriptional responses to these inhibitors.

Significance: These array data identify groups of genes synergistically affected by diacetate and lactate treatment and suggest physiological mechanisms used by *L. monocytogenes* to overcome the organic acid stress.

T2-04 High Pressure Inactivation of Noroviruses in Vegetables and Fruits

FANGFEI LOU, Hudaa Neetoo, Haiqiang Chen and Jianrong Li
The Ohio State University, Dept. of Food Science and Technology, Columbus, OH, USA

Introduction: Fresh produce is often a high-risk food because it can become contaminated at pre-harvest and post-harvest stages and it undergoes minimal or no processing. Disease surveillance shows that human norovirus ranks as the primary cause of fresh produce associated disease outbreaks (40%). Therefore, there is an urgent need to develop non-thermal processing technologies to inactivate foodborne enteric viruses in vegetables and fruits.

Purpose: The objective of this study was to determine the effectiveness of high pressure processing (HPP) to inactivate noroviruses in an aqueous medium, vegetables and fruits.

Methods: The cultivable murine norovirus was inoculated into Dulbecco's Modified Eagle Medium (DMEM), iceberg lettuce, fresh-cut strawberry and strawberry puree to a final concentration of approximately 10^7 PFU/ml or 10^7 PFU/g. The samples were treated at pressures ranging from 200 to 450 MPa for 2 min at either 4 or 20°C. The virus survivors were quantified by viral plaque assay and the inactivation kinetics of norovirus was determined.

Results: This study systematically investigated the inactivation of noroviruses in fresh produce by HPP. Murine norovirus was effectively inactivated by HPP in aqueous medium, lettuce, strawberry, and fruit puree. The pressure, pH and temperature all affected the inactivation of murine norovirus. Norovirus was more effectively inactivated at 4°C than at 20°C. Norovirus was also more sensitive to high pressure at pH 7.0 than at pH 4.0. Approximately 5 log PFU/g (or PFU/ml) reduction was achieved in all food items when pressurized at 400 MPa for 2 min at 4°C. High pressure affected the texture of lettuce and strawberries but not of strawberry puree, and did not affect the other sensory qualities such as color, aroma and freshness.

Significance: HPP effectively inactivates norovirus in fresh produce and can thus provide a novel intervention for processing fruits intended for frozen storage and fruit products such as puree, sauce, and juice.

T2-05 Phenotypic and Genotypic Characterization of Antimicrobial Resistance in *Salmonella* Serotypes Isolated from Retail Meats in Canada

MUEEN ASLAM, Valerie Bohaychuk, Gary Gensler, Richard Reid-Smith, Brent Avery and Patrick Boerlin
Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, AB, Canada

Introduction: *Salmonella* spp. are one of the most important foodborne pathogens causing severe gastroenteritis in human. Meat is considered to be one of the main sources for the transmission of *Salmonella* spp. to humans, and the occurrence of antimicrobial resistance (AMR) in *Salmonella* poses a serious public health risk.

Purpose: The objective of this study was to perform phenotypic and genotypic analysis of AMR in *Salmonella* serotypes recovered from retail meats purchased in Alberta, Canada.

Methods: A total of 564 samples comprising chicken (206), beef (134), pork (133) and turkey (91) meats were collected. *Salmonella* isolates were recovered using standard cultural methods. Isolates were confirmed using biochemical tests and PCR employing *Salmonella* specific primer sets. Serotyping was performed with standard method. A total of 326 *Salmonella* isolates were analyzed. The AMR was determined using an automated microdilution method (Sensititre® system) using Gram Negative Plates. Results were interpreted according to the Clinical Laboratory Standard Institute guidelines. PCR was used to detect resistance genes including *tet*A,B,C, *sul1,2,3*, *blaCMY-2, blaSHV, blaTEM*, *blaPSE* and *aphA1, aphA2, aadB, strA/B*.

Results: All beef samples were negative for *Salmonella*. The most common *Salmonella* serotypes identified were Heidelberg (27%), Hadar (25%) and Kentucky (14%) in chicken and turkey. AMR differed among the serotypes; 100% isolates of serovar Anatum were resistant to seven antimicrobials, including category I antimicrobials (high importance to human health by Health Canada categorization). Multiple antimicrobial resistances were also common in serovars Heidelberg, Kentucky, Infantis, Typhimurium, Typhimurium var. Copenhagen, and Kiambu. Serovars Enteritidis, Johannesburg, Montevideo, Reading, Thompson and Tennessee were sensitive to tested antimicrobials. The presence of resistance genes generally correlated with resistance phenotypes and their prevalence differed among serotypes. The *tet*C and *sul3* genes were not found in any serotype. The *blaCMY-2* gene was found in serovars Anatum (100%), Kentucky (50%), Heidelberg (30%), Infantis (30%)and Kiambu (25%). The *blaPSE* and *blaTEM* genes were found in serovars Typhimurium var. Copenhagen (40%) and Heidelberg (40%), respectively. Serovar Rissen was positive for *sul1* and *tet*B genes without phenotypically resistant to these antimicrobials.

Significance: This study provides useful information for regulatory authorities and data may be used in risk assessment studies.

T2-06 Comparison of the rfb Cluster in 16 Rare *Salmonella* Serotypes

MATTHEW L. RANIERI, Andrea Moreno Switt, Henk C. den Bakker, Martin Wiedmann, Lavorka Degoricija, Craig A. Cummings, Elena Bolchacova, Greg Govoni and Manohar R. Furtado
Cornell University, Food Science, Ithaca, NY, USA

Introduction: *Salmonella* causes an estimated 1.4 million human cases per year by foodborne transmission in the United States, and is one of the leading causes of gastroenteritis worldwide. In support of epidemiological investigations, the Kauffman-White immunologic classification scheme, which is based on somatic (O) and flagellar (H) antigens, is commonly used. To eliminate the need for hundreds of antisera, molecular serotyping approaches offer an alternative to traditional methods, and have been investigated with respect to *Salmonella* serotypes commonly associated with foodborne illness.

Purpose: The purpose of this study was to analyze the rfb locus, which encodes for the nucleotide sugar biosynthesis pathways and transferases necessary for assembly of lipopolysaccharides that produce the O-antigen, for 16 less common human disease associated *S. enterica* subsp. enterica serotypes.

Methods: Sixteen *Salmonella* isolates representing serovars (serogroup) Adelaide (O), Alachua (O), Baildon (D2), Gaminara (I), Give (E1), Hvittingfoss (I), Inverness (P), Johannesburg (R), Minnesota (L), Mississippi (G), Montevideo (C1), Rubislaw (F), Senftenberg (E4), Uganda (E1), Urbana (N), and Wandsworth (Q) were sequenced by the SOLiDTM next generation sequencing system. By *de novo* assembly, the rfb locus for each strain was identified, and individual genes were putatively identified using the Rapid Annotation using Subsystem Technology (P-RAST).

Technical

Results: Within *Salmonella* serogroups or across serogroups sharing a common antigenic factor, there was a high degree of similarity, especially with genes related to sugar biosynthesis. However, comparisons among serogroups revealed considerably less homology in gene content. One common gene, identified as a flippase, was found in the majority of serotypes, and encodes for transmembrane proteins responsible for transferring O-units across the cytoplasmic membrane.

Significance: The identification of conserved genes across rare serotypes indicates potential for the application of molecular serotyping to the serogroups investigated in this study. Identification of O-antigens with molecular methods will provide an alternative to traditional serotyping, which is labor intensive, costly, and difficult to standardize.

T2-07 Sequences in the *comK* Prophage Junction Fragments Cluster *Listeria monocytogenes* Isolates of Epidemic Clones II, III and the 2008 Canadian Outbreak into Subclones That are Unique to Individual Meat and Poultry Processing Plants

MEI LOK, Yi Chen, Bindhu Verghese, Peter Evans, Sophia Kathariou and Stephen Knabel
Penn State University, Dept. of Food Science, University Park, PA, USA

Introduction: Listeria monocytogenes epidemic clones (ECs) II and III have been associated with large multistate outbreaks due to contaminated ready-to-eat (RTE) meat and poultry products in the U.S. The Food Safety and Inspection Service (FSIS) of the United States Dept. of Agriculture (USDA)'s RTE meat and poultry products monitoring program has generated a large number of *L. monocytogenes* isolates from meat and poultry processing facilities throughout the U.S. Based on pulsed-field gel electrophoresis profiles a small fraction of these isolates appeared to be ECII. Isolates with ECII profiles were also isolated from two turkey processing plants in the U.S. (Eifert et al., 2005). However, it is not known whether specific subclones of ECII and III, and the 2008 Canadian outbreak clone were associated with individual processing plants in the U.S.

Purpose: 1) To confirm that the isolates from FSIS and Eifert et al. (2005) were ECII, 2) To determine whether or not specific subclones of ECII and ECIII, and the 2008 Canadian outbreak clone were associated with individual plants and 3) To determine if *comK* prophages in ECII and ECIII can spontaneously induce, form phage and are infective or defective.

Methods: ECII PCR, multi-virulence-locus sequence typing (MVLST), comK prophage PCR, comK prophage junction fragment sequencing, attP and attB PCR, phage DNA PCR and plaque assay were performed.

Results: ECII PCR and MVLST confirmed that the isolates from FSIS and Eifert et al. (2005) were ECII. *comK* prophage PCR showed that most ECII isolates produced the same results as strains from the two known ECII outbreaks, except six FSIS isolates. *comK* prophage junction fragment sequencing identified subclones, most of which were unique to individual processing plants. *attP* and *attB* PCR revealed that the *comK* prophages in ECII and III could be spontaneously induced. Phage DNA PCR and plaque assays suggested that ECIII formed defective phages containing *comK* phage DNA.

Significance: Public health agencies can use *comK* prophage junction fragment sequencing, in conjunction with MVLST, to trace subclones of *L. monocytogenes* to identify reservoirs, sources and transmission pathways. This information will allow implementation of more effective intervention strategies for controlling *L. monocytogenes*. Further research is needed to determine whether the genes in the *comK* prophage junction fragments play a role in colonization and persistence of these unique subclones in individual plants, as well as in their transmission to foods and humans.

T2-08 An ABC Transporter Regulates Biofilm Formation by Controlling the Expression and Modification of Cell Surface Proteins in *Listeria monocytogenes*

Xinna Zhu, Weibing Liu, René Lametsch, Frank M. Aarestrup, Chunlei Shi, Qunxin She, XIANMING SHI and Susanne Knøchel
Dept. of Food Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

Introduction: Listeria monocytogenes is a foodborne pathogen that can form biofilms in food processing environments. In a previous study, the lm.G_1771 gene encoding an ABC-transporter permease is involved in negative regulation of biofilm formation in *L. monocytogenes* 4b G by analyzing genetically a Tn917 insertion mutant LM-49 (phenotype: biofilm-forming enhanced).

Purpose: The aim of this study was to reveal the possible mechanism of this ABC-transporter permease controlling biofilm formation in *L. monocytogenes* by characterization of a Δ1771 mutant with lm.G_1771 gene deleted.

Methods: The ability of biofilm formation by the deletion mutant Δ1771 was performed using violet crystal staining assay. The characterization of the Δ1771 mutant was assessed by AI-2 bioluminescence assay, antibiotics assay and Triton X-100 induced autolysis. The differential proteome and gene expression between the wide-type strain and the mutant Δ1771 was compared using two-dimensional (2D) gel electrophoresis with combining mass spectrometry and complementary DNA (cDNA) Microarray, respectively.

Results: The Δ1771 mutant had shown the same enhanced ability of biofilm formation as LM-49. An AI-2 bioluminesence assay demonstrated that the Lm.G_1771 permease did not export the AI-2 signaling molecule, suggesting that the regulation must involve a novel signal transduction pathway. Functional proteomic/genomic analyses identified several differentially expressed proteins/genes in an lm.G_1771 deletion mutant, and these included 15 proteins revealed by 2D protein gel electrophoresis and 48 genes identified from transcriptomic analyses. For the Δ1771 mutant, the differential expression genes that increased in the Δ1771 mutant were related to cell envelope (six genes), stress response (two genes), protein secretion (three genes), transcriptional regulator (three genes), and unknown function (eight genes), and the repressed genes included the dlt operon and many genes with unknown function.

Significance: Our results suggest that the lm.G_1771-mediated signal transduction pathway regulates *L. monocytogenes* biofilm formation by controlling the expression of biofilm-mediating proteins and those involved in their modification.

T2-09 Maximizing Personnel Hygiene, Minimizing Washroom Contamination

John Holah and DEB L. SMITH
Campden BRI, Gloucestershire, United Kingdom

Introduction: Contamination of food by food workers has been identified as an important contributing factor during foodborne illness investigations. Hands must be washed prior to food handling, though the efficacy of such handwashing, and how to prevent the recontamination of the handwash user and the washroom environment during handwashing and drying have been little studied.

Purpose: This study assessed the microbiological efficacy of a novel automated handwash system as compared to a traditional handwash technique and assessed the impact of these techniques on the microbiological contamination of the user and the environment. Similar studies were undertaken for a range of hand drying techniques.

Methods: The efficacy of an automated hand wash system was assessed against a standard UK National Health Service (NHS) handwash protocol in a traditional sink using the methodology of BS EN 1499:1997 (hygienic biocidal handwash test) and 35 volunteers. The generation of large (ballistic) and small (aerosolized) water droplets and their associated microbiological components by these techniques, and their impact on the handwash user and the washroom environment, was measured using moisture indicator paper and microbiological air sampling. Similar droplet and microbial aerosol distribution studies were undertaken for a novel air knife hand drying system, a hot air hand dryer and paper towels using 30 volunteers.

Results: A 30 s standard NHS handwash achieved a 2.42 log reduction which was statistically different from the 2.67 log order reduction achieved by the automated handwash unit (30 s). Both techniques gave rise to microbiological contamination of the environment and user (including areas of the sleeves and body likely to come into contact with food), though the automated hand wash facility gave rise to much lower levels than the traditional sink. All hand drying techniques generated insignificant microbial aerosols though water droplet contamination of the environment was significantly greater for the air dryers than for paper towels.

Significance: The automated hand wash facility demonstrated a statistically significant improvement in hand wash efficacy over the UK approved NHS manual hand wash. Recontamination of the user and the washroom environment can be minimized by the hygienic design of automated handwash facilities and the correct siting of hand drying equipment.

T2-10 Developing a Fish Starter Culture Fermentation with a Local Nisin-producing Bacterium for Application in Small-scale Artisan Fishery Production in Senegal

MICHEL B. DIOP, Jacqueline Destain, Maïmouna Cisse, Ababacar S. Ndoye and Philippe Thonart
Ministère des Mines, de l'Industrie, de la Transformation Alimentaire des Produits Agricoles et des PME, Direction de la transformation alimentaire, Dakar, Senegal

Introduction: In Senegal, fish is the main animal protein source for populations. Traditional fish production, known to have some inherent food safety problems due to the high susceptibility of seafood to bacterial spoilage, is predominant because of the lack of financial resources that hamper the development of industrial preservation technologies.

Purpose: The safety problems are particularly acute in traditionally fermented fish products in which the catches, generally handled for many hours at ambient temperature, are preserved only by adding salt (NaCl) and sun drying.

Methods: Spontaneous fermentation assayed at 30°C for 10 h on fish (*Podamassys jubelini* and *Arius heudeloti*) purchased at a local market, led to the proliferation of enteric bacteria (that can include pathogenic bacteria) to over 10^8 CFU/g. Putrid odors developed after 5 to 6 h of incubation at 30°C and became very pronounced after 24 h. When these fish were supplemented with glucose (1%, wt/wt) and inoculated with a culture of a nisin-producing *Lactococcus lactis* (10^7 CFU/g), which was previously selected from a Senegalese fermented millet (*Pennisetum glaucum*) flour, the pH decreased to about 4.60 after 10 h at 30°C. Nisin activity was detected in juice from the two fish.

Results: In the new fermentation conditions, enteric bacteria contamination was reduced to 10^3 CFU/g. The putrid odors were not very perceptible in the products, indicating the inhibition of the enteric bacteria.

Significance: The results show the potentialities of this strain, justifying a more in-depth investigation into the use of millet flour as a carbohydrate source for the starter development in the perspective of its further application in small-scale fermented fish production in Senegal.

T2-11 Characterization of a Fish-specific Monoclonal Antibody

YI-TIEN CHEN and Yun-Hwa Peggy Hsieh
Florida State University, Nutrition, Food, and Exercise Sciences, Tallahassee, FL, USA

Introduction: Fish is one of the eight major allergen foods under the Food Allergen Labeling and Consumer Protection Act. Currently, there is no convenient method for fish detection to protect sensitized individuals and enforce the labeling law. Immunoassay is known as a simple and rapid method for detection of various food ingredients. In this study, we report a fish-specific monoclonal antibody (MAb), 8F5, which was raised against crude protein extract of cooked red snapper for the development of an immunoassay for fish protein detection.

Purpose: The specific objective of this study is to characterize the species specificity and antigenic protein of MAb 8F5.

Methods: Soluble proteins of all samples were extracted by 0.15 M NaCl. The species specificity of MAb 8F5 was screened against raw and cooked (100°C, 15 min) extracts from 55 common food fish species, 14 non-fish species and 4 food additives using an indirect enzyme-linked immunosorbent assay (ELISA). Protein extracts from raw and cooked (5, 10, 15 min at 100°C) extracts of four selected fish species were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted with MAb 8F5 to reveal the antigenic component.

Results: MAb 8F5 belongs to IgG2a subclass. It strongly reacted with cooked extracts of all fish species tested without any cross-reactivity with land animals and food additives. The SDS-PAGE and immunoblot results showed that the antigenic component recognized by MAb 8F5 being a single protein with apparent molecular weight of 36 kDa. This antigenic protein maintained its molecular integrity up to 15 min of cooking suggesting the thermal-stability of this protein. In addition, the band intensity also increased with the increase of cooking time.

Significance: The fish-specific MAb 8F5, therefore, has great potential to be employed in an immunoassay for rapid and accurate detection of fish tissue in raw and cooked food products.

Technical

T2-12 Characterization of Food and Clinical *Listeria monocytogenes* Isolates Collected in Portugal

JOANA BARBOSA, Vânia Ferreira, Rui Magalhães, Isabel Santos, Gonçalo Almeida, Paul Gibbs and Paula Teixeira
Escola Superior de Biotecnologia-Universidade Católica Portuguesa, CBQF, Porto, Portugal

Introduction: The high incidence of *Listeria monocytogenes* in foods and the high fatality rate associated with listeriosis, makes this pathogen of high concern to the Food Industry. The collection and characterization of *L. monocytogenes* isolates from food are essential to a better understanding of the distribution of the pathogen through the food chain and the potential contribution of specific strains to human infection.

Purpose: We thus characterized both food and human clinical isolates of *L. monocytogenes* collected through 2003 to 2008 in Portugal. In this study 3698 *L. monocytogenes* isolates from different food products, namely vegetables (30), dairy (782), fish (19), pre-cooked meals (240), Ready-to-Eat (RTE, 217), and meat products (2410) and 75 *L. monocytogenes* isolates from human cases were investigated.

Methods: Isolates were tested for (i) the major serotype-specific genes: serotypes 1/2a and 3a (subtype A), serotypes 1/2b, 3b and 7 (subtype B), serotypes 1/2c and 3c (subtype C), serotypes 4b, 4d and 4e (subtype D) and serotypes 4a and 4c (subtype E); and (ii) resistance to arsenic (Ar), cadmium (Cd) and tetracycline (Tet).

Results: Subtype A isolates were most frequently detected on vegetables (46.7%), dairy (38.4%) and pre-cooked meals (92.9%) products; subtype B was most frequently detected on fish (57.9%) product isolates, and subtype D was most frequently detected on RTE (39.6%) and meat products (45.9%) isolates as well as on clinical isolates (68.0%). The most frequent profiles for Ar, Cd and Tet among isolates from different food products was as follows: ArSCdSTetS on vegetables (73.3%), dairy (54.9%) and clinical isolates; ArSCdRTetS on fish (47.4%), pre-cooked meals (80.0%), RTE (43.3%) and meat products (55.2%). Interestingly, profiles ArRCdSTetR and ArSCdRTetR were only detected on dairy and meat products isolates, respectively.

Significance: Overall, our data suggested that specific characteristics of isolates might be associated with their source.

T3-01 Prevalence and Distribution of *Salmonella* in Organic and Conventional Broiler Poultry Farms

WALID ALALI, Siddhartha Thakur, Roy D. Berghaus, Michael Martin and Wondwossen A. Gebreyes
University of Georgia, Center for Food Safety, Griffin, GA, USA

Introduction: Poultry is known to be an important vehicle of *Salmonella* transmission to humans, mainly via contaminated meat. Production of organic poultry in the U.S. has grown considerably to meet market demand in the past two decades. Very little is known, however, about the prevalence and dissemination of *Salmonella* in USDA-certified organic chicken farms.

Purpose: The objective of this cross-sectional study was to compare the prevalence of *Salmonella* and antimicrobial-resistant *Salmonella*, as well as investigate the distribution of this pathogen in organic and conventional broiler poultry farms.

Methods: Fecal, feed, and water samples were collected from birds at 3 weeks and 8 weeks of age for 2-flock cycles. One house was sampled per farm at 3 organic and 4 conventional broiler farms from the same company in North Carolina. All samples were analyzed for the presence of *Salmonella* using selective enrichment techniques. Further phenotypic (antimicrobial susceptibility) and genotypic (PFGE) testing were performed.

Results: The overall prevalence (across all farms and sample types) of *Salmonella* in organic farms was 4.3% (13/300) compared to 28.8% (115/400) in conventional farms. *Salmonella* prevalence in fecal samples were 5.6% (10/180) and 38.8% (93/240) from organic and conventional farms, respectively. From feed, 5% (3/60) and 27.5% (22/80) of the samples were positive for *Salmonella* from organic and conventional farms, respectively. None of the water samples were positive for *Salmonella*. Antimicrobial susceptibility testing was performed on 70 representative *Salmonella* isolates (12 from organic farms and 58 from conventional farms). In isolates from organic farms, 25% (3/12) were pansusceptible, 33.3% (4/12) had single antibiotic resistance (mostly to streptomycin), and 41% (5/12) were multi-drug resistant. In conventional farms, 1.7% (1/58) of the isolates were pansusceptible, 36.2% (21/58) were single-antibiotic resistant, and 62% (36/58) were multi-drug resistant; 43% (25/58) of the multi-drug resistant isolates were resistant to six drugs. Characterization of the 72 isolates using PFGE showed a high clonal nature of the isolates within and among the two types of farms.

Significance: The results of our study suggest that within this poultry company, prevalence of fecal *Salmonella* may be lower in certified-organic birds than in conventionally-raised birds. In addition, the prevalence of antimicrobial resistant *Salmonella* appears to be higher in conventionally-raised birds than in certified-organic birds.

T3-02 Molecular Surveillance of Multi-antibiotic Resistant *Staphylococcus aureus* and *Salmonella* Isolated from Co-op Rabbit and Poultry Processing Plants in Southeastern United States

LEONARD WILLIAMS and Olasunmbo Ajayi
North Carolina A&T State University, Center of Excellence for Post Harvest Technologies, Kannapolis, NC, USA

Introduction: The distribution practices and changes in food processing, by which potentially contaminated food could reach consumers across multiple geographic regions, presents a new and complicated challenge for public health and food safety experts in the United States.

Purpose: A study was conducted to determine the prevalence and genetic diversity of multi-antibiotic resistant strains of *Salmonella* and *Staphylococcus* spp. isolated from co-op rabbit and poultry processing plants in five states (Florida, Alabama, Tennessee, Mississippi and Georgia).

Methods: Phenotypic characteristics, antimicrobial and genetic relationship were analyzed in one-hundred and two *Salmonella* and *Staphylococcus* isolates collected from 360 broiler and rabbit samples from four sampling points (pre- and post-evisceration; pre- and post-chilling) from commercial broiler and rabbit processing facilities. Biochemical and antimicrobial analysis using 16 different antibiotics and API strips were used to determine the biochemical patterns among the isolates. Polymerase chain reaction and pulsed field gel electrophoresis analysis were performed to detect methicillin (mecA) and clumping factor (cflA) gene for *Staphylococus aureus* and fimbrin (fimA) gene for *Salmonella* and the genetic relatedness of the strains using SmaI restriction enzyme and analyzed using Bionumeric software program.

Results: Results indicated that ten different biochemical profiles were detected among the 53 (15%) *Staphylococcus aureus* and 60 (18%) of *Salmonella* isolates recovered from the broiler and rabbit samples. Isolates of distinct phenotypic profiles were detected within a sampling point. Fifty-three (100%) isolates were positive for clfA gene, 19 (36%) for mecA gene and 19 (30%) for fim A gene using PCR. Antibiotic testing revealed that of the 113 *Staphylococcus* and *Salmonella* strains tested, 67% of the strains were sensitive, while 63% were resistant to multiple antibiotics. Both *Salmonella* and *Staphylococcus* isolates were all typed by pulsed field gel electrophoresis, which identified 8 different types of *Salmonella* and 4 different types of *Staphylococcus aureus*. Type A and its subtypes comprised of
45 and 30% of all *Salmonella* and *Staphylococcus aureus* isolates recovered from post-evisceration sampling points, respectively.

Significance: Similar strains were prominent among several co-op processing facilities, suggesting possible cross-contamination and a combination of molecular or antimicrobial typing tools maybe an effective epidemiological tool for source tracking in meat processing facilities.

T3-03 Prevalence and Antimicrobial Resistance of *Campylobacter* Isolated from the National Antimicrobial Resistance Monitoring System Retail Meat: 2002—2007

SHAOHUA ZHAO, Linda English, Jason Abbott, Emily Tong, Niketta Womack, Sharon Friedman and Patrick McDermott
U.S. Food and Drug Administration, Laurel, MD, USA

Introduction: Campylobacter is a leading cause of foodborne diarrhea illness. Antimicrobial resistance in *Campylobacter* spp. from the food supply is a global public health concern.

Purpose: The objectives of this study were to determine the prevalence, antimicrobial susceptibility, and genetic relatedness of *Campylobacter* spp. recovered from the National Antimicrobial Resistance Monitoring System (NARMS) retail meat program.

Methods: We compared the prevalence of *Campylobacter* in a sampling of 24,566 meats including chicken breast (n = 6,138), ground turkey (n = 6,109), ground beef (n = 6,171) and pork chop (n = 6,148) from ten FoodNet sites collected during 2002—2007. Isolates were further speciated by PCR and analyzed for antimicrobial susceptibility by agar dilution or broth microdilution and compared genetic relatedness using pulsed-field gel electrophoresis (PFGE).

Technical

Results: A total of 2,258 of *C. jejuni*, 925 *C. coli* and 7 *C. lari* were identified. Chicken breast showed the highest contamination rate (49.5%), followed by ground turkey (1.6%), and both pork chops and ground beef had < 0.5% of contamination. Resistance was most frequent to doxycycline/tetracycline (46.6%), followed by nalidixic acid (18.5%), ciprofloxacin (17.4%), azithromycin and erythromycin (2.8%), telithromycin (2.4%), and clindamycin (2.2%) and gentamicin (< 0.1%). All isolates were susceptible to meropenem and florfenicol. With exception of doxycycline/tetracycline, *C. coli* showed higher resistance to all other antimicrobials than *C. jejuni*. PFGE fingerprinting profiles showed that *Campylobacter* were genetically diverse with 1,226 PFGE profiles generated from the 2318 isolates. Several clones were found to be geographically dispersed and to persist throughout the six-year sampling.

Significance: Results demonstrated a high prevalence of antimicrobial-resistant *Campylobacter* in chicken meat, emphasizing the importance of sustained monitoring of the food supply.

T3-04 The Effect of Heat on the Antimicrobial Efficacy of Cinnamic Aldehyde, Carvacrol and Eugenol

GRISHMA KOTWAL and Faith J. Critzer
University of Georgia, Food Science and Tech., Griffin, GA, USA

Introduction: Several natural compounds have been evaluated to determine their antimicrobial capacity; however, research is needed to study the different parameters that affect the activity of these compounds in order to determine the conditions that may alter efficacy and help establish appropriate use in foods.

Purpose: The objective of the study was to quantify the antimicrobial activity of three phytophenolic essential oil components (cinnamic aldehyde, carvacrol and eugenol) after exposure to various temperature conditions, against *Escherichia coli* O157:H7 and *Salmonella enterica* serovars.

Methods: Pure aromatic volatiles (≥ 98% purity) in black micro centrifuge tubes were subjected to temperature exposure for 0 h, 0.5 h, 1h, 4h, at 60°C and 70°C, and for 0 h, 12 h, 24 h, 48 h, 72 h, 7 d, 14 d and 21 d at 4°C and 25°C. The in vitro determination of antimicrobial activity was carried out through the microbroth dilution assay. Concentrations of 1, 2, 5, and 10 mM of each antimicrobial were evaluated in order to determine the Minimum Inhibitory Concentration (MIC) against five strains of each foodborne pathogen independently.

Results: The MIC results for the untreated antimicrobial components showed carvacrol to be the most effective with an MIC of 1mM, followed by cinnamic aldehyde MIC = 2 mM and eugenol MIC = 5 mM. The MIC increased to 2, 5 and 10 mM for carvacrol, cinnamic aldehyde, and eugenol, respectively, when samples were held at 70°C for 4 h. A slight reduction in activity was observed in carvacrol with exposure ≥14 days (MIC = 2 mM) and cinnamic aldehyde ≥ 21 days (MIC = 5 mM) when held at 4°C and 25°C, while eugenol retained activity at these temperatures.

Significance: It is important to establish efficacy of antimicrobial compounds in a wide array of processing conditions. The retention of antimicrobial activity by these naturally occurring essential oil components will allow flexibility when processors seek to incorporate them into food. Further work is being conducted to determine the activity of these compounds in a model milk system.

T3-05 Cinnamaldehyde Induces Cell Elongation in *Escherichia coli* O157:H7

Richard A. Holley and VISVALINGAM JEYACHCHANDRAN
University of Manitoba, Food Science, Winnipeg, MB, Canada

Introduction: Plant essential oils and their components have been widely studied for their ability to inhibit pathogenic and spoilage organisms in foods. One such compound, cinnamaldehyde, is active against many foodborne pathogens including *Escherichia coli* O157:H7; however its mechanism of antimicrobial action is not fully understood. It has been suggested that cinnamaldehyde may damage the cell membrane and inhibit polymerization of the cell septum protein ftsZ.

Purpose: To examine changes in growth and cell morphology of *E. coli* O157:H7 during exposure to sub-lethal concentrations of cinnamaldehyde.

Methods: Log phase *E. coli* O157:H7 (02/0627) cells were treated with 100, 200 or 300 mg/l cinnamaldehyde (< minimal inhibitory concentration, 400 mg/l) at 37°C for 4 h. Growth was monitored by hourly plate count and absorbance (A_{600}) while filament formation and cell viability were monitored by photomicroscopy after 5- cyano-2, 3- ditolyl tetrazolium chloride (CTC) and Live/Dead BacLight vitality staining. Mean cell length values, the number of viable cells and differences in growth response were compared by ANOVA.

Results: Cinnamaldehyde at ≤ 200 mg/l significantly ($P < 0.05$) delayed *E. coli* O157:H7 growth, causing a ≤ 2 h lag while 300 mg/l prevented growth for 4 h. The greatest extent of filamentation (94.7%) and greatest mean cell length (6.2 µm) occurred at 2 h exposure to 200 mg/l cinnamaldehyde. After 2 h at 200 mg/l, changes to normal cell length and growth rate were noted. Cell viability by vital staining was unaffected by ≤ 200 mg/l cinnamaldehyde, but after 1 h at 300 mg/l, only 75 % of cells were viable, 30% of cells were still filamentous, and little change was noted for the next 3 h.

Significance: At sub-lethal concentrations, cinnamaldehyde caused a delay in *E. coli* O157:H7 replication because of filament induction which was reversible at ≤ 200 mg/l.

T3-06 Can Hand Hygiene Regimens Offer Reduced Risk in Food Service Environments?

SARAH EDMONDS, Christopher Fricker and David Macinga
GOJO Industries, Research and Development, Akron, OH, USA

Introduction: The FDA Food Code specifies that food handlers must maintain clean hands by washing with soap and water. Alcohol-based hand sanitizers (ABHS) may be used in conjunction with hand washing, but are not allowed as a substitute for hand washing. There is currently limited data to demonstrate the added antimicrobial benefit of a wash-sanitize regimen in comparison to hand washing with soap and water only.

Purpose: To compare the antimicrobial activity of a wash-sanitize regimen as compared to the standard hand wash with soap and water. To evaluate the impact of product formulation (handwash or ABHS) on antimicrobial efficacy.

Methods: A modification of ASTM E1174 was used. Hands were contaminated with approximately 1×10^9 *Escherichia coli* suspended in chicken broth to replicate a moderate food soil. Eighteen subjects participated for each of the six test configurations described below. Log_{10} reductions from baseline were calculated for each configuration. Statistical analyses were performed using an ANOVA ($P < 0.05$).

Results: The log_{10} reductions for each configuration are as follows: non-antimicrobial handwash (3.10 ± 0.61), antimicrobial handwash (3.56 ± 0.74), non-antimicrobial handwash followed by ABHS foam (3.81 ± 0.89), antimicrobial handwash followed by ABHS foam (4.16 ± 0.91), non-antimicrobial handwash followed by advanced formula ABHS gel (5.13 ± 0.71), and antimicrobial handwash followed by advanced formula ABHS gel (5.22 ± 0.60). When used individually, the non-antimicrobial handwash and antimicrobial handwash were statistically equivalent. All wash-sanitize regimens were statistically superior to any type of hand washing regime. Wash-sanitize regimens incorporating the advanced formula ABHS gave statistically significantly higher efficacy than those incorporating the ABHS foam.

Significance: Hand washing is an appropriate hand hygiene intervention in low risk environments (i.e., clearing tables), however a wash-sanitize regimen can provide an additional antimicrobial benefit and should be considered for high risk situations such as handling raw meat.

T3-07 SaniTwice™: A Hand Hygiene Solution for Reducing Contamination on Heavily Soiled Hands When Water is Unavailable

SARAH EDMONDS, Robert McCormack, Christopher Fricker and David Macinga
GOJO Industries, Research and Development, Akron, OH, USA

Introduction: The FDA Food Code specifies that food handlers must clean their hands by washing with soap and water. However, food is frequently prepared and served in areas where clean water or sinks are unavailable. SaniTwice, a two-step hand hygiene procedure using an alcohol-based hand sanitizer (ABHS), has been shown to be an effective option for reducing bacteria on lightly soiled hands in low to no water situations. However, the effectiveness of SaniTwice against bacterial contamination on heavily soiled hands is currently unknown.

Purpose: Evaluate the antibacterial efficacy of the SaniTwice method on hands in the presence of heavy food soils.

Methods: A modification of ASTM E1174 (Health Care Personnel Handwash Test Method) was used. Hands were contaminated by either handling raw hamburger or cooked chicken chunks seeded with *Escherichia coli* to represent heavy soils found in food service settings. Two test product configurations were evaluated with each soil: a non-antimicrobial handwash and SaniTwice using a 62% ABHS foam. The SaniTwice procedure involved “washing” with an excess of ABHS and paper towel drying; followed by reapplication of ABHS according to label instructions. Five participants evaluated each of the four configurations and $\log_{10}$ reductions from baseline were calculated for each configuration.

Results: When hands were contaminated by handling chicken chunks, the non-antimicrobial handwash and SaniTwice achieved $\log_{10}$ reductions of 2.96 ± 0.48 and 3.32 ± 0.43, respectively. When hands were contaminated by handling raw hamburger, the non-antimicrobial handwash and SaniTwice achieved $\log_{10}$ reductions of 2.58 ± 0.41 and 2.69 ± 0.34, respectively.

Significance: The SaniTwice method is an effective option for hand hygiene when handwashing resources are unavailable, as it achieved bacterial reductions on heavily soiled hands that are equivalent to traditional hand washing.

T3-08 Analysis of Plasmids and Mobile Elements Carrying Antimicrobial Resistance Genes in *Salmonella* Isolates by Whole Genome Sequencing

ANDREA I. MORENO SWITT, Matthew Ranieri, Henk den Bakker, Lovorka Degoricija, Craig A. Cummings, Gregory Govoni, Elena Bolchakova, Manohar Furtado and Martin Wiedmann
Cornell University, Dept. of Food Science, Ithaca, NY, USA

Introduction: Foodborne salmonellosis causes an estimated of 1.4 million human cases annually in the United States. Plasmids carrying antimicrobial resistance genes have been described among diverse *Salmonella* serovars. Furthermore, dissemination through the food chain of plasmids encoding resistance genes is a global public health concern.

Purpose: The purpose of this study was to identify the repertoire of antimicrobial resistance genes in mobile elements among diverse human disease related *Salmonella* serovars by full genome sequencing.

Methods: Sixteen *Salmonella* isolates representing serovars Senftenberg, Rubislaw, Gaminara, Hvittingfoss, Minnesota, Urbana, Alachua, Adelaide, Wandsworth, Johannesburg, Baildon, Mississippi, Inverness, Montevideo, Uganda and Give were sequenced by the SOLiD next generation sequencing system.

Results: By *de novo* assembly, putative large plasmids were identified among the genomes. In the serovar Montevideo isolate an IncW plasmid was detected carrying sulfonamide and aminoglycoside resistance genes in an integron class 1. This element includes a cluster of genes homologous to a previously sequenced plasmid of a multidrug resistant *Salmonella* Newport isolate. No other isolates harboring plasmid associated resistance genes were identified. However, in serovar Inverness, we identified one large IncI1 plasmid of approximately 120 kb, and one 64 kb putative integrated conjugative element. In addition, we found putative IncI1 plasmids lacking resistance genes, but with characteristics of conjugative plasmids in serovar Mississippi and Urbana isolates.

Significance: Plasmids carrying antimicrobial resistance genes and mobile elements with self transmissible features were identified using full genome sequencing of *Salmonella*. Identification of a number of plasmids without resistance genes suggests a larger repertoire of transmissible genetic elements in *Salmonella* than previously recognized; these elements may allow for emergence of new plasmids carrying antibiotic resistance genes.

T3-09 Considering the Design and Analysis of Efficacy Trials for Antimicrobial Treatments of Raw Meat and Poultry

MARK POWELL
USDA, Washington, D.C., USA

Introduction: Recent policy developments have spurred interest in the design and interpretation of experiments to evaluate the efficacy of antimicrobial treatments for raw meat and poultry products. Current domestic and international guidelines for approval of antimicrobial treatments differ markedly and provide limited guidance on statistical design and analysis of efficacy trials. Determining whether a treatment effect exists for the entire target population is complicated when results vary among replicate trials (e.g., days or plants).

Purpose: The study compares the statistical power of an illustrative antimicrobial efficacy experiment under the standard analysis of variance (ANOVA) model with fixed effects to that under a mixed model with fixed treatment and random replicate effects.

Methods: Statistical power is analyzed for an experimental design with three treatment groups: (1) no treatment, (2) treatment without antimicrobial additive (e.g., water-only wash), and (3) treatment with antimicrobial additive; and three replicate trials. To optimize power, the design plans for two independent contrasts: (1) no treatment versus treatment (with or without additive) and (2) treatment without additive versus treatment with additive. Compared to no treatment, it is anticipated that treatment without additive averages a 0.5 $\log_{10}$ reduction in microbial contamination, and the antimicrobial treatment averages a 1 $\log_{10}$ reduction, with antimicrobial efficacy varying among replicates. Because standard procedures do not consider such variation, power for the mixed model is estimated using bootstrap sampling.

Results: The power of the test of the incremental antimicrobial effect is lower under the mixed model, and the disparity increases with variance among replicates; however, inferences from the standard ANOVA cannot be generalized due to heterogeneous treatment efficacy among replicates.

Technical

Significance: The reduced power under the mixed model illustrates the inherently greater challenge of generalizing from limited experimental conditions to the entire population. For a fixed total sample size, mixed model power increases with the number of replicates (fewer samples per trial), but this comes at the expense of inferential power within replicates that may help explain the observed heterogeneity.

T3-10 Evaluation of a Predictive Model for Total Viable and Lactic Acid Bacteria on Refrigerated Vacuum-packed Beef Primals

MARK TAMPLIN, Alison Small, Sheila Peddell, Olivia McQuestin, Bianca Porteus, Danielle O'Callaghan, John Sumner and Ian Jenson
University of Tasmania, Food Safety Centre, Sandy Bay, TAS, Australia

Introduction: The shelf-life of fresh beef is affected by intrinsic and extrinsic factors including temperature, pH, packaging atmosphere and microbial species. The storage life of meat can be controlled, in part, by understanding the kinetics of microbial growth that contributed to spoilage. Such information can be converted into predictive models that aid in managing cold chains.

Purpose: The purpose of this study was to produce and validate a predictive model for the viability of total viable count (TVC) and lactic acid bacteria (LAB) on vacuum-packed beef primals.

Methods: TVC and LAB models were produced by studies of microbial growth on commercially-packed primal cuts, and separately for cuts irradiated and then inoculated with a 6-strain spoilage cocktail. Microbial counts and surface pH were measured at selected time intervals on samples stored at -1.5, 0, 2, 4 and 7°C for up to 30 weeks. Growth parameters were estimated for lag time, growth rate and maximum population density, and then transformed into secondary models. Model estimates were compared to independent data of growth rates in the published literature, ComBase and industry data.

Results: The model based on laboratory-inoculated meat over-estimated TVC and LAB levels when compared to data in ComBase and the published literature. For example, the bias and accuracy factors were 2.8 and 3.1 for TVC, respectively. Producing the model from commercially-packed primal cuts improved bias and accuracy factors to 1.6 and 2.8 for TVC, respectively. However, growth rate predictions for TVC and LAB were still 3 to 4 times higher than the rates observed for whole beef primals produced by six Australian abattoirs and stored at -0.5°C.

Significance: These findings demonstrate that microbial communities, and their interactions, are likely complex in fresh beef, and that these differences may affect the validity of model applications for commercial products.

T3-11 Inactivation of *Bacillus coagulans* Spores in Tomato Juice by Pressure-assisted Thermal Processing

HOSSEIN DARYAEI, Chelsea Johnson and V.M. (Bala) Balasubramaniam
The Ohio State University, Food Science and Technology, Columbus, OH, USA

Introduction: *Bacillus coagulans* is a heat-resistant spore-forming bacterium, spoiling thermally-sterilized acidic foods. Pressure-assisted thermal processing (PATP) is a promising technology capable of inactivating bacterial spores and producing shelf-stable food products. Lesser thermal effects due to uniform compression heating, as well as rapid cooling, allows better quality preservation in PATP comparing to conventional processing.

Purpose: This study was aimed to evaluate the efficacy of PATP for inactivation of *B. coagulans* spores in tomato juice.

Methods: A spore crop of *B. coagulans* 185A (selected as the most pressure-heat resistant strain amongst three strains) was prepared on nutrient agar plus 500 mg/L dextrose and 3 mg/L $MnSO_4$. H_2O, incubated at 50°C for 7 d. Spores were suspended (4.2×10^8 CFU/mL) in a commercial tomato juice (pH 4.2) and treated at 600 MPa and process temperatures of 95, 100 and 105°C for ≤ 2.5 min. The inactivation data was fitted to estimate kinetic inactivation parameters using selected linear and non-linear models. The models were evaluated using the mean square error, regression coefficient and accuracy factor.

Results: Depending upon the process temperature, a 600 MPa PATP treatment reduced the spore population by 3.0—3.6 logs during the 30 s pressure come-up time. Treatment times of 2.5, 2 and 0.5 min were sufficient to inactivate the spores to below the detection limit (< 10 CFU/mL) at 95, 100 and 105°C, respectively. Pre-process temperature history also significantly influenced ($P < 0.05$) PATP-induced spore inactivation. Kinetic model analysis indicated that the non-linear models produced better fits than the linear model to all survivor curves.

Significance: This study suggests that combined pressure-heat treatment may be used as a viable alternative to inactivate *B. coagulans* spores in acidic food products, like tomato juice.

T3-12 Non-thermal Pasteurization of Almonds and Pistachios with Organic Citrus Bioflavonoid Extracts

PEYMAN FATEMI, Cliff Coles and Yves Methof
Aurora Food Safety Solutions, Pleasanton, CA, USA

Introduction: Thermal treatments of nuts have been widely used because of lack of effective alternative pasteurization technologies. There is a need for non-thermal treatments that will achieve the minimum 4-log reduction of *Salmonella* without impacting the sensory attributes of nuts. Antimicrobial properties of citrus bioflavonoids have been used in the meat and other industries. To be effective, treatments must overcome the natural surface barriers of nuts.

Purpose: Objective of this work was to evaluate effectiveness of organic citrus bioflavonoid extract (BioSecur 428D) in improving the safety of nuts by combining this technology with an electrostatic spray method that created negatively charged 30—40 µm droplets for better nut surface coverage.

Methods: Whole almonds or pistachios were inoculated with either *Enterococcus faecium* or *Salmonella* Enteritidis Phage Type 30 per protocols of the Almond Board of California. Twenty-five g samples were added to sterile bags and rinsed or electrostatically sprayed with equal amounts of treatment at various time and concentration combinations. Controls included un-rinsed nuts as well as those rinsed/sprayed with water alone. Controls indicated that rinsing/spraying with water alone for up to 5 minutes did not remove *E. faecium* from the nut surfaces. Therefore, any reduction of bacteria levels was due to the action of treatment.

Results: Rinsing/spraying the almonds and pistachios with BioSecur 428D was effective in achieving greater than 5-log reduction of *E. faecium* and *Salmonella* SEPT30. Lower treatment concentrations required longer exposure times of up to 5 minutes while higher concentrations achieved greater than 6-log reduction after 1 minute of exposure. Overall, these results show that it is possible to achieve the target microbial reduction on nuts using citrus extracts at concentration and exposure times applicable to industrial settings.

Significance: Improve the safety of nuts. Introduce a novel and cost effective approach in antimicrobial treatments. Application to other commodities.

T4-01 Field Assessment of Surface Contamination and Systemic Transference of an Attenuated *Salmonella* Typhimurium to Melon Fruit from Controlled Contamination of Irrigation Water

TREVOR SUSLOW, Adrian Sbodio, Alejandro Tomás-Callejas, Francisco López-Gálvez and Sharyn Maeda
University of California-Davis, Plant Sciences, Davis, CA, USA

Introduction: Establishing microbiological quality standards for irrigation water, within food safety management programs for fresh produce is a contentious topic. The California melon industry requested the initiation of a study on the potential for systemic transfer of *Salmonella* to mature fruit in a field environment.

Purpose: Evaluate the survival and dispersal of applied *Salmonella* Typhimurium following controlled irrigation-source contamination events in an open field environment.

Methods: Cantaloupe ('Oro Rico') and Honeydew ('Summer Dew') in replicated plots in Davis, CA (Reiff Coarse Loam) were irrigated, by furrow or sub-surface emitter tape, containing a rifampicin resistant (100 mg/L) isolate of virulence-attenuated *Salmonella*. "Contamination" levels were targeted at log 4 and 6 CFU/ml of water applied. At several periods, samples of water, furrow and seed-bed soil, rhizosphere soil, soil surrounding the emitter tape, and environmental samples were processed by standard protocols including selective enrichment and molecular confirmation. Mature fruit in the central bed (crown) as well as fruit developing in contact with furrow slopes were processed, with and without disinfection with mercuric chloride (10 min + 1 min sterile water rinse), as above.

Results: Applied *Salmonella* Typhimurium was readily recovered from irrigation inputs, soil, and environmental samples throughout the trial. The applied strain was regularly recovered from cantaloupe but not honeydew developing in "contaminated" furrows. No *Salmonella* was recovered from the rind or in the sub-rind tissue taken below the abscission zone in over 200 crown fruit. The applied *Salmonella* survived in the rhizosphere of melons irrigated by sub-surface drip injection but no evidence of transfer to fruit was detected.

Significance: These results support greenhouse outcomes in our lab that strongly suggest a very high threshold of contamination from irrigation water would be required to make the risk of root uptake and transfer to fruit a significant concern. External contamination of fruit remains a plausible direct or indirect route from irrigation water.

T4-02 Tracking an *Escherichia coli* O157:H7 Contaminated Batch of Leafy Greens through a Commercial Processing Line

ANNEMARIE L. BUCHHOLZ, Gordon R. Davidson, Rudolph E. Sloup, Bradley P. Marks, Ewen C. Todd and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: Cross-contamination of uncontaminated leafy greens with small amounts of *Escherichia coli* O157:H7-contaminated product during commercial processing has been suggested as one cause for recent outbreaks.

Purpose: The goal of this study was to quantify the spread of *E. coli* O157:H7 during small-scale commercial processing of iceberg lettuce, occurring via transfer within the system or by transport of contaminated particles.

Methods: Radicchio (9.1 kg) was dip-inoculated to contain 10^6 CFU/g of a 4-strain avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 cocktail. The processing line consisted of a commercial shredder, step conveyor, flume tank, shaker table and centrifugal dryer. After priming this line with 45 kg of iceberg lettuce, the inoculated Radicchio was processed and immediately followed by 907 kg of iceberg lettuce (triplicate experiments). Forty bags of lettuce/Radicchio (~22.7 kg/bag), 40 water samples (500 ml) and 50 equipment swabs (100 cm^2) were targeted for collection. All visible shreds of Radicchio were retrieved from these 40 bags, the equipment, and the floor and were weighed and counted. Twenty-five grams of only iceberg lettuce was collected from each bag and from 10 of the 40 bags after centrifugal drying. All samples were examined for *E. coli* O157:H7 by direct plating or membrane filtration with trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin used as the growth medium.

Results: After processing, an average of 614.92 g (93.64%), 6.93 g (1.25%), 5.00 g (0.78%) and 2.85 g (0.47%) of inoculated Radicchio appeared in the shredded lettuce from bags 1 to 10, 11 to 20, 21 to 30 and 31 to 40. These same bag groupings contained average *E. coli* O157:H7 counts of 1.69, 1.22, 1.10 and 1.11 log CFU/g, respectively, with most of this contamination coming from the water and equipment surfaces. No significant decrease in *E. coli* O157:H7 was seen in the iceberg lettuce after centrifugal drying. Hundreds of Radicchio pieces were recovered from the equipment surfaces after processing with the conveyor harboring the most in terms of weight (9.79 g) followed by the shredder (8.32 g), flume tank (3.53 g) and shaker table (0.08 g).

Significance: Based on these findings, *E. coli* O157:H7-contaminated leafy greens are able to perpetuate in a processing line long after a contamination event, and thereby contaminate large quantities of previously uncontaminated product.

T4-03 Recovery of *Escherichia coli* O157:H7 from Inoculated Spinach Fields as Affected by Inoculum Dose, Plant Material and Environmental Conditions

EDUARDO GUTIÉRREZ-RODRÍGUEZ, Steven Koike, Michael Cahn and Trevor Suslow
University of California-Davis, Plant Science, Davis, CA, USA

Introduction: Leaf colonization by epiphytic bacteria and human pathogens has been described as irregular and dependent on phyllosphere characteristics and environmental conditions. Bacterial attachment is influenced by physicochemical properties of bacteria and the leaf being colonized. Extreme variability in bacteria populations within leaves reduces the efficacy of sampling and the ability of detection methods to minimize distribution of contaminated product.

Purpose: To determine the heterogeneity of persistence on spinach leaves over time by *E. coli* O157:H7 under field conditions following foliar inoculation.

Methods: Spinach was cultivated in the Salinas Valley, California from June to November 2009. Spinach was sprayed with log 0.3 and 0.56 CFU/m^2 of a mixture of two attenuated strains. Individual leaves and composites up to 150 g were used in the recovery of *E. coli* O157:H7 with and without disinfection with 1% $AgNO_3$. Quantitative recovery was by selective and differential culture. Assurance GDS-O157™ was used for qualitative detection of *E. coli* O157:H7 below the limit of plating recovery.

Results: Composite 150 g sample units consisted of 165 to 220 leaves. Average populations after 12 to 36-h post-inoculation were 1.4 and 3.1 CFU/g, respectively. Fifty percent of plants inoculated with log 0.56 CFU/m^2 and 25% of those from low dose were positive for *E. coli* O157:H7 after 6 days post-inoculation. On average, populations recovered from both treatments were of 1.35 CFU/g and remained constant in all trials over 28 days. Recovery of *E. coli* O157:H7 from 30 individual leaves was 35% and 57% (lower dose and higher dose, respectively) without disinfection, while 8% and 17% after disinfection.

Technical

Significance: Our findings indicate that even homogeneous contamination events result in heterogeneous distribution over time. Pathogen sampling protocols for leafy greens should be standardized for sample mass units greater than the standard 25 g from raw or processed samples due to the significant variability observed in the surviving populations.

T4-04 Molecular Ecology of *Listeria* spp., *Salmonella*, *Escherichia coli* O157:H7, and Non-O157 Shiga Toxin-producing *E. coli* in Northern Colorado Wilderness Areas

CHRISTINA AHLSTROM, Clyde Manuel, Kelly Horgan, Martin Wiedmann and Kendra Nightingale
Colorado State University, Animal Science, Fort Collins, CO, USA

Introduction: Listeria monocytogenes, Salmonella, Escherichia coli O157:H7, and non-O157 shiga toxin producing *E. coli* (STEC) represent clinically important foodborne pathogens in the U.S. A knowledge gap exists regarding the presence, transmission and molecular ecology of these foodborne pathogens in pristine environments

Purpose: The objective of this study was to collect a set of foodborne pathogens from pristine environments to aid in interpretation of a shared subtype between clinical and food isolates during an outbreak.

Methods: Five wilderness locations in Colorado were selected to represent pristine environments and three areas approximately 100 m^2 within each location were designated; where each area was sampled during the spring, summer, and fall of 2009. A total of 225 soil, 225 water, 45 drag swab and 117 fecal samples were collected. Up to five soil samples and five water samples from each area were pooled. The average pH of soil samples was 5.2 ± 0.6, which may be explained by sampling coniferous forest soil overlaid by decomposed acidic needles. Furthermore, all samples were microbiologically analyzed to detect *Listeria* spp., *Salmonella, E. coli* O157:H7, and non-O157 STEC. After non-selective pre-enrichment, samples were divided and analyzed to detect each target organism using a modified version of the Food and Drug Administration Bacteriological Analytical Manual. Up to four presumptive colonies for each target organism from each sample were confirmed by PCR to detect gene fragments specific to those organisms.

Results: Overall, two samples tested positive for *L. monocytogenes,* including one soil and one fecal sample. Eight samples were positive for *Listeria* spp. other than *L. monocytogenes*, including four fecal, one water and three soil samples, which were all determined to be *L. welshimeri* via *sigB* sequencing. No *E. coli* O157:H7 or *Salmonella* were detected.

Significance: Our results demonstrate a rare presence of foodborne pathogens in pristine environments and further characterization of these isolates will provide insight into epidemiological associations between isolates in nature and clinical isolates.

T4-05 Development of a Simple Method to Detect Coliphages in Fresh Produce as Evidence of Fecal Contamination

CHOI-IOK R. WONG and Fu-Chih Hsu
Scientific Methods Inc., Granger, IN, USA

Introduction: The consumption of ready-to-eat produce has become a major source of foodborne outbreaks in the USA because it is especially prone to contamination that may go unchecked and result in severe illness. Due to the difficulty of identifying potential pathogens that may be present in contaminated fresh produce, reliance on indirect measures of contamination, such as the presence of indicator microorganisms, becomes critical to protect public health. Monitoring traditional bacterial indicators such as *E. coli*, along with other alternative indicators like coliphages as viral indicators, may provide more food safety measures.

Purpose: In this study, a simple method was developed to detect male-specific (F+), somatic coliphages, and *E. coli* from ready-to-eat produce.

Methods: The method included a pectin-based medium containing chromogenic substrates, bacterial stain and an elution buffer for the recovery of coliphages from vegetables and fruits. A modified membrane filtration method was used to detect *E. coli* from washes.

Results: In spiking experiments, the recoveries of F+ coliphages from washes using the pectin medium were 72% (lettuce), 44% (strawberries), 48% (baby carrots), 29% (cherry tomatoes), and 58% (green onions). In a field study, 181 samples were collected from retail stores or farmers markets in the USA. Of the 181 field samples tested, 81.2% were positive for *E. coli*, 21.3% were positive for F+ coliphages, and 35.7% were positive for somatic coliphages with ranges of 0 to 3×10^6 CFU/100 g (*E. coli*), 0 to 1.3×10^4 PFU/100 g (F+ coliphages), and 0 to tntc (somatic coliphages). Among vegetables analyzed, alfalfa sprouts, ready-to-eat baby spinach, and cilantro tested positive for all fecal indicators with higher concentrations and more frequent than other vegetables tested.

Significance: The results from this study show that it is feasible to use the pectin-based medium to detect coliphages in vegetable washes collected from markets. The coliphage results may provide additional safety measures for ready-to-eat produce along with other bacterial indicators such as *E. coli*.

Technical

T4-06 Internalization of Murine Norovirus-1 to Romaine Lettuce

JIE WEI, Yan Jin, Tom Sims and Kalmia E. Kniel
University of Delaware, Animal and Food Sciences, Newark, DE, USA

Introduction: Norovirus is a leading cause of foodborne illness associated with consumption of fresh produce. Irrigation with surface water such as river or canal water has resulted in increasing interests concerning virus internalization into plants through root tissue. Little is known on the uptake of enteric viruses into leafy greens.

Purpose: To study the likelihood of virus internalization into Romaine lettuce through root tissue, lettuce sprouts were measured over time for virus viability and infectivity.

Methods: Five-day old Romaine lettuce sprouts were fed 1×10^7 or 1×10^4 PFU murine norovirus-1 (MNV) daily for up to three days. MNV was pipetted at the roots carefully without contact to shoots. After one or three days, shoots were collected and blended with a tissue homogenizer. The samples were then applied to cell culture to determine the concentration of infectious virus, or viral RNA was extracted and applied to reverse transcription and quantitative PCR (RT-qPCR). MNV antiserum was produced by specific pathogen-free birds and IgY was purified with aluminum sulfate. Shoots were stained with chicken anti-MNV IgY and observed under confocal microscopy to evaluate the presence of MNV. Control uninoculated sprouts in the presence of Hanks' Balanced Salt Solution were grown or assessed identically in each experiment.

Results: For both day one and day three incubation, in two out of three trials, shoots samples incubated with 1×10^7 PFU MNV contained ~ 5 log RT-qPCR units MNV; however, the cell culture assay showed that only 1 to 1.5 log PFU MNV was infectious. While the sprouts incubated with 1×10^4 PFU MNV, no virus was detected in either day one or day three samples. MNV was observed in both stem and leaf of the sprout incubated with 1×10^7 PFU MNV for one day under confocal microscopy. No virus was visible with the lower inoculation or control samples.

Significance: This is the first study to show that MNV could internalize into lettuce sprouts through the root. Current data suggests that this could occur when high numbers of virus are present, i.e., a flood or sewage leak.

T4-07 Hydroponic Internalization of Enteric Viruses into Green Onions and Spinach

KIRSTEN A. HIRNEISEN and Kalmia E. Kniel
University of Delaware, Animal and Food Science, Newark, DE, USA

Introduction: Enteric viruses including both hepatitis A virus (HAV) and human noroviruses have been the cause of outbreaks involving both green onions and leafy greens. During the washing of fruits and vegetables, internalized pathogens evade removal and inactivation by sanitizers. Internalization of non-viral pathogens through intact roots has received much debate. Previous studies on viruses indicated limited internalization into plants grown in soil.

Purpose: This study addresses the internalization of enteric viruses into spinach and green onion plants when grown in hydroponic solution contaminated with HAV and a common norovirus surrogate, murine norovirus (MNV).

Methods: Spinach and green onion sprouts were grown in Hoagland's solution containing 0.2% bacto agar, inoculated with HAV (7 log TCID50) or MNV (5.5 log PFU). After seven days, plants were washed with Virkon® to inactivate viruses present on outer surfaces prior to detection by qPCR infectivity studies. Survival of viruses in soil and homogenized spinach and green onions was determined by qPCR or by TCID50 and plaque assay in mammalian cell culture.

Results: Virus persistence and internalization were evaluated. HAV persisted in soil for 90 days; qPCR data indicated a 2.35 ± 0.7 log genomic copies/g reduction in titer. Both HAV and MNV were able to persist in homogenized green onion and spinach tissue for 10 days with no significant reduction in titer as shown by TCID50 and plaque assay ($P < 0.05$). When grown in hydroponic solution, 6.05 ± 0.12 log genomic copies of HAV and 3.25 ± 0.23 log PFU of MNV were internalized into spinach. Similarly, 4.08 ± 0.5 PFU of MNV was internalized into green onions.

Significance: Many commercial crops are grown hydroponically. HAV and MNV internalize and persist within spinach and green onion tissues increasing the potential for contamination. This study increases the knowledge of virus-plant interactions and provides information useful for risk assessment.

T4-08 Controlled Environment Assessment of Preharvest Internalization and Transference of *Salmonella* into Melon Vines from Irrigation Water Using a Tube Nucleation Assay

CAROL B. D'LIMA, Kin H. Tan and Trevor V. Suslow
University of California-Davis, Davis, CA, USA

Introduction: Melon vines harbor endophytes, including enteric bacteria that may enter fruit at some point in maturation. The risk of systemic transfer of pathogens from roots to fruit needs clarification.

Purpose: Evaluate potential for dose-dependent root uptake of *Salmonella* in controlled environments by tube nucleation assay (TNA).

Methods: Cantaloupe ("Oro Rico" F1) vines were irrigated at 4—5 leaf stage with a single inundative treatment, at various doses, of *S. Poona*, transformed with the *ice*C gene. Inoculum was applied to the rhizosphere by sub-irrigation. "Contamination" levels were uninoculated, log 2, 4, 6 and 8 CFU/ml of water applied. Successive internodal sections of log 8 CFU/ml irrigated plants were also analyzed to determine the extent of acropetal systemic movement. Following surface sterilization, detection of up to 80 vines/treatment was conducted by TNA, confirmed by culture and molecular techniques.

Results: Recovery of internalized *Salmonella* after one day was detectable within 0, 7, 57 and 96 percent at log 2, 4, 6 and 8, respectively. After one week 14% of the vines inoculated at the highest concentration were positive for internalization. *Salmonella* was detected among 90 percent of the basipetal internodal segments among vines analyzed on Day 1. Detection was reduced to 10 percent after 2 weeks. Analysis of successive internodal sections on Day 1 showed a decrease to 30% recovery in the adjoining section, dropping to 10% in the top most section. Basipetal sections analyzed after 2 weeks were consistent with earlier outcomes (< 10% positive).

Significance: These model system experiments indicate that, upon a contamination event of a high dose of *Salmonella* into the soil via irrigation water, there exists the high potential for immediate, but apparently transient, transfer of enteric pathogens into melon vines. Systemic transference to fruit must be considered. However, results indicate that there is a very high threshold of contamination from irrigation water to make this plausible.

T4-09 The Effect of Total Organic Carbon Content and Repeated Irrigation on the Persistence of *Escherichia coli* O157:H7 on Baby Spinach

DAVID T. INGRAM, Cheryl Mudd, Sean Ferguson, Dallas Hoover, Kalmia E. Kniel and Manan Sharma
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: In response to U.S. foodborne illnesses caused by contaminated spinach, growers have adopted regulations stated in the California Leafy Greens Marketing Agreement (LGMA). The LGMA permits a maximum population mean of 126 Most Probable Number (MPN) generic *E. coli* per 100 ml irrigation water. These metrics, however, do not include other measurements of water quality that might enhance the epiphytic survival of bacterial pathogens.

Purpose: We investigated the effect of repeated irrigation of baby spinach plants with water containing differing levels of total organic carbon (TOC) on the epiphytic survival of enterohemorrhagic *E. coli* O157:H7 (EHEC).

Methods: Sterile, diluted bovine manure was prepared to contain TOC concentrations of 0ppm, 12 — 15 ppm and 120 — 150 ppm. An inoculum of three nalidixic acid-resistant EHEC strains was introduced into each irrigation solution at either a high (5—6 log CFU/100 ml) or low (0—1 log CFU/100 ml) population, and sprayed twice a week onto baby spinach plants using a fine mist air-brush. A 3-tube MPN was used to determine the persistence of EHEC on the aerial tissues, which were harvested daily.

Results: Plants irrigated with high EHEC populations, regardless of TOC levels, showed a 3-log reduction within the first 24 h. EHEC then exhibited low levels of persistence for up to 16 days on all treatments, ranging from 76.4 MPN per plant (day 1) to 0.40 MPN per plant (day 16). For the irrigation events containing low EHEC populations, no viable EHEC were detected after 24 h. The TOC content of irrigation water did not affect the persistence of EHEC on baby spinach tissues.

Significance: This study suggests that EHEC populations in irrigation water which comply with LGMA guidelines will not survive for more than 24 h on foliar surfaces of spinach plants. The TOC content of irrigation water did not influence EHEC survival.

T4-10 Effect of Modified Atmosphere on Persistence and Virulence Expression of *Escherichia coli* O157:H7 on Shredded Lettuce

Sudesna Lakshman, Sean Ferguson, David T. Ingram, Jitendra R. Patel, Yaguang Luo and MANAN SHARMA
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: Fresh-cut lettuce contaminated with *Escherichia coli* O157:H7 has caused multiple foodborne outbreaks. Packaging and storage temperature may affect the persistence and ability of the pathogen to cause illness.

Purpose: To evaluate packaging atmospheres and storage temperatures on the behavior of *E. coli* O157:H7 on shredded lettuce.

Methods: Shredded lettuce was inoculated with 4.07 CFU/g of nalidixic-acid resistant *E. coli* O157:H7, and packaged into: gas permeable films with an initial oxygen level set at 2 Pka (treatment A), micro-perforated films (treatment B) or non-gas permeable films (treatment C). Inoculated and uninoculated samples were stored at 4 or 15°C for up to 10 days. Populations of *E. coli* O157:H7 and aerobic heterotrophs were enumerated on MacConkey and tryptic soy agar, respectively. *E. coli* O157:H7-RNA from inoculated lettuce at 15°C was analyzed by reverse transcriptase real-time PCR for expression of 5 virulence factors (VF) on each day - *stxII*, *eae*, *iha*, *rfbE*, and *espA*. Two replicate experiments were performed.

Results: No significant ($P < 0.05$) differences in *E. coli* O157:H7 populations were observed among the three packaging atmospheres at 4°C. At 15°C, *E. coli* O157:H7 populations under treatment B were significantly lower than other treatments on days 7 and 10. Heterotrophic populations from uninoculated lettuce under treatment B were significantly greater than populations from other treatments on day 3 at 4°C and day 1 at 15°C. Expression of *stxII*, *eae*, *iha*, and *rfbE* under treatment B was significantly greater than under other treatments on day 7, but significantly lower than under treatment C on day 10.

Significance: Different packaging atmospheres affected the persistence and virulence factor expression of *E. coli* O157:H7 on lettuce at 15°C, as well as heterotrophic populations at 4 and 15°C. Packaging atmospheres of fresh-cut lettuce may affect the ability of *E. coli* O157:H7 to cause illness.

T4-11 Inactivation of *Escherichia coli* O157:H7 on Spinach and Parsley Using Low-energy X-ray Irradiation

SCOTT R. MOOSEKIAN, Sanghyup Jeong, Bradley P. Marks and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: Several multistate *Escherichia coli* O157:H7 outbreaks in the United States have been linked to consumption of baby spinach and parsley. These outbreaks highlight the inability of standard commercial washing practices to reduce bacterial pathogens to safe levels on fresh produce, re-emphasizing the need for improved microbial inactivation strategies.

Purpose: In this study, low-energy X-ray irradiation was investigated to inactivate *E. coli* O157:H7 on the surface of baby spinach and flat-leaf parsley.

Methods: Baby spinach leaves obtained at retail were round-cut (5.07 cm^2) using a sterile cork borer, whereas single flat-leaf parsley leaves were removed from the stem and digitally imaged with these pixilated images factored into a computer algorithm to determine the leaf surface area. Both products were dip-inoculated in a 3-strain cocktail of *E. coli* O157:H7 (K3995 - 2006 spinach outbreak, K4830 - 2006 lettuce outbreak A, K4492 - 2006 lettuce outbreak B) (9.43 log CFU/ml) to obtain ~5.8 log CFU/cm^2 on the leaves. After 24 h of storage at 4°C, triplicate samples were processed in a prototype low-energy X-ray irradiator (Rayfresh Foods, Ann Arbor, MI). Each side of the sample was irradiated to achieve five combined surface doses of up to 0.176 kGy for spinach and 0.205 kGy for flat-leaf parsley, with these dose levels confirmed using radiochromic film dosimeters. *E. coli* O157:H7 survivors were quantified in sample homogenates by plating appropriate dilutions on Sorbitol MacConkey Agar containing cefixime and tellurite (no evidence of sublethal injury seen in previous work), followed by incubation at 37°C for 24 h.

Results: The analyses yielded D_{10}-values of 0.035 and 0.052 kGy for *E. coli* O157:H7 inoculated spinach and parsley, respectively. These are similar to the value reported for iceberg lettuce (0.039 kGy using low-energy X-ray). Given the enhanced efficacy of X-ray irradiation, the dose required to achieve a 5 log reduction (0.175 and 0.260 kGy) would be significantly lower than the FDA maximum allowable dose of 4 kGy for spinach and lettuce.

Significance: Based on these findings, low-energy X-ray irradiation appears to be an effective means for inactivating *E. coli* O157:H7 on spinach and parsley, and is also likely applicable to other leafy green vegetables.

T4-12 Inactivation of *Salmonella* on Tomato Surfaces Using Gaseous Chlorine Dioxide Treatment

BASSAM A. ANNOUS, Angela Burke and Joseph E. Sites
U.S. Dept. of Agriculture, ARS-ERRC, Food Safety Intervention Technologies, Wyndmoor, PA, USA

Introduction: Tomatoes have been implicated in fourteen outbreaks of salmonellosis in the U.S. since 1996. Previous research in our laboratory has documented the inadequacy of washing processes to inactivate and/or remove microorganisms on tomatoes, including human pathogens, due to biofilm formation and inaccessibility of microbial attachment sites to washing systems.

Purpose: The objective was to develop gaseous chlorine dioxide (ClO_2) treatment capable of inactivating human pathogens attached to inaccessible sites within biofilm on the tomato surfaces.

Methods: Tomatoes were inoculated with *Salmonella* Poona RM 2350 to an approximate final concentration of 5 log CFU/g, and stored at 4°C for 24 h prior to treatment. Tomatoes were fumigated with ClO_2 for up to 6 h in a closed chamber that was developed at ERRC, using two different technologies for generating ClO_2. Following treatment, residual (non-injured and injured) populations of *Salmonella* Poona on tomatoes were enumerated using XLT-4 selective agar and TSA overlaid with XLT-4 agar media, respectively.

Results: There was in excess of 4.5 log CFU/gm reduction in *Salmonella* Poona populations following ClO_2 treatment for 6 h. Population reductions following ClO_2 treatment were similar irrespective of the technology used to generate ClO_2. The treatment helped increase the shelf life of the tomatoes by reducing the spoilage microorganism populations on the surface, and did not seem to have adverse effects on the quality of this commodity.

Significance: The work presented here showed that gaseous $CClO_2$ treatment of tomatoes was able to inactivate *Salmonella* Poona cells attached to inacessible sites such as the stem scar area. Also, this treatment was shown to extend the shelf life at 4°C and had no adverse effects on the quality of the tomatoes.

Technical

T5-01 Time-temperature Dependent Growth Patterns of *Salmonella* spp. in a Model Food System with Natural Microflora

AMIT MOREY, Shelly R. McKee and Manpreet Singh
Auburn University, Dept. of Poultry Science, Auburn, AL, USA

Introduction: The USDA-FSIS *Salmonella* Initiative Program requires post-chill temperatures to be below 40°F as a critical control point to reduce *Salmonella* prevalence in raw poultry products. Alternative parameters to regulations on time and temperature during chilling of poultry have potential to achieve the food safety objective that the FSIS is pursuing. Models predicting *Salmonella* growth at abused temperatures are developed using sterile medium without competitive microflora, while studies on growth dynamics of the pathogen at temperatures ranging from 4 to 10°C (40 to 50°F) are of particular interest to poultry processors, yet lacking.

Purpose: Experiments were conducted to study the growth of *Salmonella* Typhimurium and Heidelberg at various temperatures in brain heart infusion broth (BHI) and non-sterile chicken slurry (CS).

Methods: Nalidixic acid (60 ppm) resistant *Salmonella* Typhimurium and Heidelberg were inoculated (3 log CFU/mL) separately in non-sterile CS and BHI. The inoculated medium (5 mL/well) was dispensed in a 12-well plate (5 wells each of BHI and CS; and 2 wells as negative control) and maintained at 4, 7 and 10°C. Samples were taken every 24 h for 6 days, spread plated on XLT4 agar and Naladixic acid and incubated at 37°C for 24 h. Simultaneously, inoculated CS was incubated at 37°C and sampled every h for 18 h. Bacterial enumerations were reported as log CFU/mL. Analysis of variance was conducted to determine significant differences in growth patterns at different temperatures ($P < 0.05$).

Results: Populations of *S.* Typhimurium and Heidelberg in CS and BHI were not different ($P > 0.05$) over a 6-day period at all temperatures. Significant differences ($P < 0.05$) in the populations of *S.* Typhimurium and Heidelberg were observed at 4°C as compared to 7 and 10°C, irrespective of the medium. At 37°C, populations of *S.* Typhimurium and Heidelberg increased from 2 to 9 log CFU/mL in CS with natural flora over 18 h period.

Significance: Growth patterns of *Salmonella* at near refrigeration temperatures during carcass chilling can be useful to develop mathematical models and help processors determine that they are maintaining process control.

T5-02 Analysis of ALLRTE and RTE001 Sampling Results for *Salmonella* Species, Calendar Years 2005—2008

Kristina E. Barlow, Stephen W. Mamber, TIM B. MOHR and Evelyne Mbandi
USDA-FSIS, Washington, D.C., USA

Introduction: Salmonella may survive in underprocessed meat and poultry products (e.g., beef jerky) or contaminate Ready-to-Eat (RTE) meat and poultry products from the production environment (e.g., raw materials, food handlers, or animal vectors). In addition to *Listeria monocytogenes*, RTE meat and poultry products samples from the Food Safety and Inspection Service's (FSIS) ALLRTE and RTE001 sampling programs are tested for *Salmonella* species. ALLRTE is a random sampling program for all types of RTE products, while RTE001 is a risk-based sampling program that targets post-lethality exposed RTE products.

Purpose: To obtain, tabulate and analyze ALLRTE and RTE001 sampling results for *Salmonella* collected over a 4-year period.

Methods: The FSIS analyzed results of *Salmonella* testing of meat and poultry product samples collected under the ALLRTE and RTE001 sampling programs for calendar years 2005 through 2008. Samples were tested using methods in the FSIS Microbiology Laboratory Guidebook.

Results: The analyses included 11,823 ALLRTE samples collected from 1,989 establishments and 33,277 RTE001 samples from 2,556 establishments in calendar years 2005 through 2008. Results showed low incidences of *Salmonella*-positive samples from the ALLRTE and RTE001 sampling program, with 8 and 14 positives, respectively over the study time period. Positive product results averaged 0.07% for ALLRTE samples (range, 0 to 0.13%) and 0.04% for RTE001 samples (range, 0.01% to 0.08%). Similarly, percentages of establishments with *Salmonella*-positive samples ranged from 0 to 0.27% for ALLRTE and from 0.07 to 0.54% for RTE001. Three types of products (i.e., head cheese, pork barbecue and sausage products) accounted for about half of all *Salmonella*-positive samples.

Significance: This detailed examination of *Salmonella* species data from the ALLRTE and RTE001 sampling programs will help guide changes in policies, regulations, inspection procedures and enforcement actions relevant to the prevention of *Salmonella* contamination in RTE products.

T5-03 Molecular Characterization and Serotyping of *Salmonella* Isolated from the Shell Egg Processing Environment

MICHAEL MUSGROVE, Karen L. Liljebjelke, Kimberly D. Ingram and Arthur Hinton
USDA-ARS, Egg Safety and Quality Research Unit, Athens, GA, USA

Introduction: Salmonellosis may be contracted by the consumption of raw or undercooked eggs. In order to develop effective sanitation practices it is helpful to understand the location of *Salmonella* reservoirs in processing environments. Shell egg processing reservoirs for *Salmonella* have not been well characterized, previously.

Purpose: A study was conducted to determine which equipment and facility surfaces in the shell egg processing environment were contaminated with Salmonella. Isolates were further characterized to determine serotype and relative molecular similarity.

Methods: On 7 visits, 35 surfaces were swabbed individually using sterile gauze pads soaked in 10 mL of phosphate buffered saline. Each sample was subjected to a cultural procedure to recover *Salmonella*: pre-enrichment in buffered peptone overnight at 37°C, selective enrichment in TT and RV broths at 42°C for 18—24 h, selective plating (BGS and XLT-4) incubated at 37°C for 18—24 h, biochemical reactions (LIA and TSI slants) incubated at 37°C for 18—24 h,and confirmed using poly-clonal anti-sera. Isolates were re-streaked three times and saved prior to subsequent analyses. Isolates were sero-typed by the Kauffman-White scheme. DNA from each isolate was harvested and purified using a commercial kit. DNA was analyzed by repetitive *Enterobacteriaceae* palindrome polymerase chain reaction using an automated system. Scatter plots and dendrograms were created to visualize DNA similarity.

Results: Salmonella serotypes recovered were Braenderup (1), Kentucky (2), Heidelberg (12), Typhimurium Copenhagen (Tyco-1), and Typhimurium (7). *Salmonella*-positive surface locations were wash tanks (Kentucky, Typhimurium, Tyco), drains (Braenderup, Heidelberg), and post-wash equipment surfaces (Heidelberg, Kentucky). All Typhimurium were recovered from the wash tanks at a single rep and were similar (95%). Drain isolates were similar regardless of rep (90%). Almost 70% of the isolates were recovered from wash tank equipment surfaces from 2 reps. However, drains were a *Salmonella* reservoir in 4/7 reps.

Significance: Wash tanks and drains were important *Salmonella* reservoirs in the shell egg processing environment. These results indicate that more effective sanitation is needed for washing equipment surfaces and drains.

T5-04 Persistent and Sporadic *Listeria monocytogenes* Strains in Fermented Meat Sausage Processors

VÂNIA FERREIRA, Joana Barbosa, Kitiya Vongkamjan, Andrea Moreno Switt, Tim Hogg, Paul Gibbs, Paula Teixeira and Martin Wiedmann
Escola Superior de Biotecnologia, CBQF, Porto, Portugal

Introduction: Persistence of *Listeria monocytogenes* in food-associated environments represents a key factor for contamination of foods with this pathogen. While several authors attempted to identify specific characteristics that confer a better survival and resistance capability of *L. monocytogenes* strains in the food-processing environment, including biofilm formation and resistance to disinfectants etc., little attention is being given to the potential contributions to persistence of prophages and phage resistance.

Purpose: To identify persistent as well as transient strains associated with production of fermented meat sausages in Northern Portugal, samples of raw material and finished product from 7 processors were collected at multiple dates either at retail establishments or at processing plants. We also investigated whether presence of lysogenic prophages in *L. monocytogenes* and phage susceptibility may be associated with strain persistence in the environment.

Methods: A total of 202 *L. monocytogenes* isolates were characterized by pulsed-field gel electrophoresis (PFGE) typing. A subset of 41 isolates, including (i) 19 representing strains that persisted in a given processor and (ii) 22 representing strains that did not show evidence for persistence were tested for lysogen induction. Twenty-six prophages where induced and their lytic spectrum against the 41 strains of *L. monocytogenes* was investigated.

Results: For all seven processors PFGE provided evidence for persistence of *L. monocytogenes* for 10 to 32 months. Results obtained revealed that (i) there was no significant association between lysogeny and persistence (ii) molecular serogroup D (4b, 4d, and 4e) isolates were more susceptible to phages as compared to serogroup A (1/2a and 3a) or B (1/2b, 3b, and 7) isolates, (iii) there was no evidence for differences in phage susceptibility between persistent and sporadic strains.

Significance: Our data support that while *L. monocytogenes* serotypes differ in phage resistance, phage resistance does not seem to be associated with strain persistence.

T5-05 Impact of Chlorine and Temperature on *Listeria monocytogenes* Survival Growth Behavior on Ready-to-Eat Meats

SHIOWSHUH SHEEN, Cheng-An Hwang and Vijay Juneja
USDA-ARS-ERRC, Wyndmoor, PA, USA

Introduction: Listeria monocytogenes (Lm) continues to pose a food safety hazard in ready-to-eat (RTE) meat due to potential cross-contamination. Chlorine is commonly used to sanitize processing equipment. However, Lm may survive on processing equipment surfaces, which then contaminate food products.

Purpose: The objective of this study was to characterize the behavior of chlorine-exposed Lm on RTE meat products stored at 4, 8, or 16°C.

Methods: A 2-strain cocktail of Lm serotype 4b was pre-treated with chlorine (0, 25, and 50 ppm) for one hour, and then inoculated onto RTE meat surfaces to obtain about 3.0 log CFU/g. Samples were stored at three temperatures (4, 8, and 16°C) and Lm was enumerated at frequent intervals. The lag phase and growth rate of Lm were estimated using DMFit (Combase website, Baranyi's model).

Results: Our results indicated that Lm growth was repressed by chlorine treatment. The lag phase of Lm after exposure to 0 ppm of chlorine (4.2 days) was shorter than that of Lm shocked with 25 ppm (5.4 days) and 50 ppm (6.8 days) at 4°C. The lag phase decreased with an increase in temperature. For example, at 25 ppm, lag times were 5.2, 3.8 and 2.6 days for 4, 8 and 16°C, respectively, and increased with an increase in chlorine concentration. At 16°C, lag times were 1.2, 2.6, and 4.0 days for 0, 25, and 50 ppm, respectively. However, the growth rate increased with an increase in temperature and decreased with an increase in chlorine. The growth rate and lag phase as a function of temperature and chlorine concentration can be described using a modified Ratkowsky model and a modified Zwietering model, respectively.

Significance: The results showed that the use of chlorine can suppress the growth of Lm. The predictive models developed will contribute to microbial risk assessment of RTE meats.

T5-06 Comparison of Data from FSIS Routine and Intensified Sampling Programs for *Listeria monocytogenes* from Ready-to-Eat Establishments

KRISTINA E. BARLOW, Stephen W. Mamber, Evelyne Mbandi and Tim B. Mohr
USDA-FSIS, Washington, D.C., USA

Introduction: The U.S. Dept. of Agriculture (USDA) Food Safety and Inspection Service (FSIS) samples Ready-to-Eat (RTE) meat and poultry products and processing environments for *Listeria monocytogenes* (Lm) through routine sampling (RLm) and intensified verification testing (IVT) programs. RLm sampling is performed at each RTE establishment at least once every four years, while IVT is performed in response to positive results from all Lm testing programs.

Purpose: To compare data from FSIS routine and intensified sampling programs for Lm from RTE establishments.

Methods: Samples were collected from FSIS establishments producing post-lethality exposed RTE meat and poultry products. Product samples were collected in the final packaged form. Food contact surface (FCS) and environmental samples were collected using sterile spongesicles hydrated with Dey-Engley (DE) broth. Samples were analyzed using methods in the FSIS Microbiology Laboratory Guidebook. Samples were collected proportional to establishment size (RLm) or based on investigative needs (IVT).

Results: From 2005 to 2008 a total of 135/16,284 (0.83%) of RLm samples and 486/13,241 (3.67%) of IVT samples were positive. For RLm, 7/2,633 (0.27%) of product, 38/8,945 (0.42%) of FCS and 90/4,706 (1.91%) of non-FCS environmental samples tested positive. For IVT, 68/2148 (3.17%) of product, 125/5,915 (2.11%) of FCS and 293/5,178 (5.66%) of non-FCS tested positive. From 2005 to 2008, RLm positives decreased slightly from 9/795 (1.13%) to 59/6,005 (0.98%), while IVT positives decreased from 164/3,060 (5.36%) to 135/3,681 (3.67%).

Significance: Overall, percentages of positives were highest for the IVT program, likely because of the investigative nature of IVT. For the RLm program, positives were highest for the non-FCS, followed by the FCS, and product samples. For IVT, positives were highest for non-FCS, followed by product, and FCS. FSIS uses the results from both sampling programs to identify cross contamination and harborage and to help protect public health.

T5-07 Comparison of a Novel Sample Collection Device and Cellulose Sponge for the Collection of *Escherichia coli* from Beef Carcasses

KEVIN CHURCH, Jared G. Maughan, Kelly M. Tesar and Amanda S. Vos
Microbial-Vac Systems, Inc., Jerome, ID, USA

Introduction: Current collection methods for the sampling of beef carcasses routinely employ cellulose sponges. The M-Vac sampling system was designed as an alternative to conventional methods for microbial sampling in diverse applications, including surface sampling of beef tissue. The device utilizes directed spray of sterile diluent coupled with vacuum suction to collect a sample from the surface of interest. Comparative studies of the M-Vac against the cellulose sponge are required to determine performance in collection of samples from beef carcasses.

Purpose: To evaluate the performance of the M-Vac sampling system in comparison to the cellulose sponge for collection of *E. coli* O157:H7 on artificially inoculated flank cuts from beef carcasses. A secondary objective was to determine if the sampling devices could potentially spread contamination to uncontaminated sites.

Methods: Flank cuts were obtained from hanging beef carcasses from a local slaughter facility. Duplicate 100 cm^2 sites were outlined on external surface and artificially inoculated with *E. coli* O157:H7 ATCC 700728 at either low (5.0×10^{-2} CFU/cm^2) or high (1.5 CFU/cm^2) inoculum levels. Following inoculation, flank sections were cooled (4°C) for 18 h prior to sampling. M-Vac sampling was conducted with 40 ml of Butterfield's Buffer per site. Cellulose sponges were hydrated with Butterfield's Buffer prior to sampling. Following sampling of an inoculated site, sampling devices were used to sample a secondary, non-inoculated site before processing. The secondary sites were excised using a sterile scalpel. Samples were enriched in mEHEC broth and incubated at 42°C for 18 h prior to analysis by PCR for presence/absence.

Results: The rate of positive samples for *E. coli* O157:H7 from inoculated sites was higher for the M-Vac than the sponge. Mantel-Haenszel Chi-square analysis of the data revealed a significant difference in the rate of positive samples from the artificially inoculated sites between the M-Vac and sponge at low inoculum level ($X^2 = 7.84$), but no significant difference at the high inoculum level. Excision results from secondary sampling sites showed that the sponge had a significantly higher rate of positive samples at high inoculum ($P < 0.05$), but no significant difference was observed at the low inoculum.

Significance: These data suggest that that the M-Vac may be a suitable alternative to sponge sampling of beef carcasses. The M-Vac may provide a more representative sample of surface bacteria when present in low numbers, and potentially reduce the risk of spreading contamination to different areas on the carcass.

T5-08 Three Sampling Methods to Recover Bacterial Populations on Beef Trimmings in Commercial Settings

MICHAEL DE LA ZERDA, Mansour Samadpour and Mohammad Koohmaraie
IEH Laboratories and Consulting Group, Meat Division, Lake Forest Park, WA, USA

Introduction: Escherichia coli O157:H7 is a potential pathogen in beef trimmings. Implementing good dressing procedures prevents pathogens from entering the meat supply, and along with a robust sampling and testing program can verify the efficacy the food safety system. Identification of beef trimmings contaminated with *E. coli* O157:H7 is reliant upon the execution of a robust sampling program.

Purpose: Identify a sampling method to recover a larger quantity of organisms from beef trimmings to provide sufficient opportunity to accurately detect organisms within the volume of beef trimmings available for sampling.

Methods: Forty-two combo bins of beef trimmings (85% lean) in a commercial processing facility were sampled using Excision 60 pieces (E), surface shaving (SS), and core drilling (CD). Samples were tested for Aerobic Plate Count (APC), Total Coliform Count (TCC), and Biotype I *E. coli* (ECC). Results were compared on the basis of log CFU/sample, log CFU/g, and log CFU/375 g.

Results: T-tests determined log CFU results for all comparisons were equivalent for E and CD ($P > 0.05$), while SS recovery was higher across all comparisons for all tests ($P < 0.05$). SS recovery was higher by 1.08, 0.85, and 0.58 log CFU/sample for APC, TCC, and ECC compared to the next numerically higher value recovered by CD. SS recovery was higher by 1.27, 1.04, and 0.77 log CFU/g for APC, TCC, and ECC compared to CD. SS recovery was higher by 1.27, 1.03, and 0.77 log CFU/375 g for APC, TCC, and ECC compared to CD.

Significance: Sampling method may be a limiting factor in the ability of commercial beef operations to recover a large amount of organisms in a sample. Surface shaving collected more organisms overall compared to Excision 60 pieces or core drilling, which were equivalent.

T5-09 Prevalence of *Clostridium difficile* in Various Types of Ground Meat and Poultry Products

Jeff Bussey, Clarissa Sugeng, Jeffrey M. Farber and JOHN AUSTIN
Health Canada, Bureau of Microbial Hazards, Ottawa, ON, Canada

Introduction: The increasing incidence of community-acquired *Clostridium difficile* associated disease has raised concerns over the possibility of foodborne transmission of *C. difficile*. Recent surveys of ground beef for the presence of *C. difficile* indicate its presence in up to 20% of Canadian ground beef samples and up to 50% of U.S. ground meat samples.

Purpose: The current study was undertaken to confirm the presence of *C. difficile* in Canadian ground beef and determine whether other types of ground meats may contain this organism.

Methods: Seventy-five samples of retail ground meat and poultry products were tested for the presence of *C. difficile* using selective enrichment, followed by ethanol shock and plating on selective agar. Presumptive positive colonies were confirmed as *C. difficile* by detection of L-proline aminopeptidase activity and PCR detection of the triose phosphate isomerase gene.

Results: Clostridium difficile was found in 13 of the 75 samples tested (17.3% positive). *Clostridium difficile* was detected in every type of ground meat including 4 of 22 (18%) samples of ground beef, 1 of 9 samples of veal (11%), 4 of 12 (33%) samples of ground turkey, 1 of 15 samples of ground chicken (7%), 1 of 9 samples of ground pork (11%) and 2 of 16 (13%) samples of ground lamb.

Significance: The results confirm the presence of *C. difficile* in Canadian ground beef, and are the first demonstration of *C. difficile* in Canadian ground turkey and pork. This is the first report of *C. difficile* in ground chicken and lamb.

T5-10 The Control of *Salmonella* Typhimurium in Poultry: From Vaccination to Specific Immunotherapy

Anthony Pavic, Peter Groves and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Salmonella Typhimurium has been associated with poultry as a vehicle and vector for zoonotic infection in humans. As this serovar is prevalent in both humans and poultry in Australia, poultry vaccines (killed and attenuated) have been developed against it.

Technical

Purpose: The objectives of this study were to measure the serological and bacteriological response in vaccinated hens, the yolks of their eggs, and their live progeny, to challenge with 10^4 CFU *S.* Typhimurium, and to determine whether prophylactic immunotherapy (anti-Typhimurium IgY), administered through feed, would reduce carriage of the bacterium in chicks.

Methods: Commercially available killed vaccine was injected intramuscularly at 12 (primer) and 18 (booster) weeks of age (woa) into Cobb hens, which were serologically tested every two weeks post-primer to challenge with *S.* Typhimurium at 20 woa. Post-euthanasia (at 24 woa) the blood and eggs (serological) and caeca (bacteriological) were tested for *S.* Typhimurium. The progeny of vaccinated and non-vaccinated hens were challenged (10^4 CFU/ml) at 3 days of age (doa) and, at 20 doa, blood and caeca were tested. The egg yolks from vaccinated hens were collected and tested (ELISA) for the presence of IgY. From those with high titre (>1000), crude IgY was prepared and used to resuspend dried egg yolk powder. This suspension was fed to day-old chicks (at 3% of daily nutrient intake) prior to challenge (104 CFU) at 3 doa. At 10 doa, the chicks were euthanized and caeca cultured.

Results: The serum ELISA results post-primer showed slow seroconversion (8% positive at 14 and 16 woa, 26% at 18 woa and 39% at 20 woa) which still afforded significant (X^2 $P = 0.001$) protection to vaccinated hens, based on culture of caeca. Both serum (33% positive) and egg yolk (16% positive) ELISA titres means (n = 60) were significantly different (Students *t*-Test $P < 0.05$) between vaccinated and non-vaccinated hens. While negative for circulating anti-Typhimurium IgG, the progeny from vaccinated hens showed significantly (X^2 $P = 0.047$) lower colonization rates. The IgY titres in egg yolk, crude IgY extract and supplemented feed ranged from 181 to 1890/ml, 20 to 1000/ml and 40—400/g, respectively, throughout the trial. The populations of salmonellae in caeca were no different in treated (4.5 $\log_{10}$ CFU/g) and control (4.2 $\log_{10}$ CFU/g) chicks.

Significance: The vaccine afforded protection against *S.* Typhimurium in hens, and their progeny (day-old chicks). However, the prophylactic use of crude anti-Typhimurium IgY, supplemented into poultry starter feed, did not prevent or reduce colonization in chicks.

T5-11 Enhanced Recovery of *Campylobacter jejuni* from Chick Paper under Hatchery Incubation Conditions

Jeremy W. Chenu, Anthony Pavic and JULIAN M. COX
University of New South Wales, Faculty of Science, Sydney, NSW, Australia

Introduction: Campylobacter jejuni is a foodborne human pathogen often associated with broiler (meat) chickens. The route of transmission in the broiler flock is unclear and although horizontal transmission is likely, vertical transmission may occur. Isolation from low-moisture samples using standard cultural techniques is difficult. As incubation in the hatchery may provide the ideal environment for persistence of *C. jejuni* because of its high temperature, relative humidity and CO_2 levels, such conditions may also improve recovery during analysis of environmental sources.

Purpose: To determine the recovery rates of *C. jejuni* from artificially inoculated chick paper during incubation under aerobic and enriched CO_2 (ca. 5% O_2, 10% CO_2, 85% N_2), high humidity and elevated temperature conditions.

Methods: Eighty chick paper samples (5 cm^2) were inoculated with ca. 10^2 CFU of a field isolate of *C. jejuni*. Half the samples were incubated aerobically at 38°C. The other forty samples were incubated in jars with a microaerobic atmosphere and high humidity at 38°C. Samples were removed and placed in 25 mL of 3M Tecra Campylobacter Enrichment Broth at 15 (10 samples), 30 (10 samples) and 60 (20 samples) mins. Following delayed selective enrichment, samples were streaked onto standard selective agars and screened using an ELISA.

Results: All papers were positive after 15 min. After 30 min, 9 of 10 papers were positive under modified storage whereas none were positive after aerobic storage. After 60 min, the results were 4 of 20 and 0 of 20, respectively. The ELISA and streak plating showed equivalence for the detection of positive samples.

Significance: As the hatchery pad paper dried during storage, *C. jejuni* died or became unculturable. However, under specialized storage conditions the cells were able to survive longer than under aerobic conditions. In the humid environment of the hatchery incubators, *C. jejuni* may be able to survive for a longer period than expected. Holding environmental samples under conditions emulating the hatchery may enhance recovery of *C. jejuni*.

T5-12 Validation of a High Throughput DNA Extraction and Real-time PCR Detection of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and *Listeria* spp.

WENDY LAUER and Jean-Philippe Tourniaire
Bio-Rad Laboratories, Hercules, CA, USA

Introduction: To meet the demands of labs with large sample numbers, a high throughput DNA extraction procedure was developed using a 96-well microplate format. This procedure was evaluated followed by detection of target organism by real-time PCR.

Purpose: The objective of this study was to compare iQ-Check high throughput DNA extraction protocol and real-time PCR detection to the appropriate US reference method for detection of *E. coli* O157:H7 in raw ground beef, *Salmonella* spp. in raw chicken breast, raw ground beef, raw pork and fresh spinach, *Listeria monocytogenes* in deli turkey and *Listeria* spp. on stainless steel.

Methods: Matrices were inoculated with a low level of target organism and an MPN was determined on the day of analysis. Samples were processed with the high throughput DNA extraction procedure and according to reference method specifications. Chi-square analysis was calculated according to McNemar for paired samples and according to Mantel-Haenszel for unpaired samples.

Results: At a 95% confidence level, there was no significant difference between the PCR test method and the reference method. All samples that were positive by the test method were confirmed according to reference method protocol. The PCR test was able to identify more confirmed *E. coli* O157:H7 samples than the reference method.

Significance: Sample processing can lead to a large bottleneck in a microbiology lab. A high throughput DNA extraction followed by real-time PCR can meet the demands of high volume labs.

T6-01 Pathogen Presence and Indicator Organism Levels during Turned Pile Composting of Broiler Litter and Aerated, Static Pile Composting of Mixed Feedstocks

ACHYUT ADHIKARI, Andy Bary, Craig Cogger and Karen Killinger
Washington State University, Food Science, Pullman, WA, USA

Introduction: Assessing pathogen risk associated with composting is important to understand potential pathogen introduction in produce farming.

Purpose: In an on-farm, two-year study of composting (aerated static pile-ASP composting with mixed feedstocks, including dairy manure and broiler litter, and turned pile-TP composting of broiler litter), pathogen presence and indicator organism levels were examined.

Methods: In the process to further reduce pathogens (PFRP), ASP must reach 55°C for 3 days and TP for 15 days with 5 turns. Piles were built each year; five locations were sampled at three depths (surface, 30 cm, 60 cm); and homogeneous samples were collected after turning or moving. TP was sampled at initiation, turnings, moving and field application. ASP was sampled twice after meeting PFRP, moving and field application. Samples were quantified for fecal coliforms and generic *Escherichia coli* (*E. coli*), and analyzed for pathogen presence (*E. coli* O157 and *Salmonella*).

Results: For TP, at initiation through turn 3, fecal coliforms and generic *E. coli* in samples collected after turning were higher than samples collected at the surface, 30 and 60 cm before turning. Generic *E. coli* at all depths were < 0.5 log MPN/g dw. In Year 1, pathogens were detected at initiation, and turns 2, 3, and 5. In Year 2, *E. coli* O157 was detected at turns 2 and 5, and after meeting PFRP. For ASP, pathogens and generic *E. coli* were not detected in Year 1. Fecal coliforms remained below the detectable limit until moving (2.7 log MPN/g dw) and field application (4.7 log MPN/g dw). In Year 2, fecal coliforms at the surface remained above 3.0 log MPN/g dw. At field application, fecal coliforms were 3.5 log MPN/g dw and generic *E. coli* were 0.47 log MPN/g dw. Pathogens were detected in the initial feedstocks and at two sampling periods after meeting PFRP.

Significance: Pathogens were detected in samples with low generic *E. coli levels*. In Year 2, pathogens were detected in composted broiler litter and mixed feedstock compost after meeting PFRP using two composting methods. Pathogens were not detected at field application in this study.

T6-02 Produce Microbial Quality is Associated with Surface Microbial Contamination in Packing Sheds: An Assessment of Risk Factors for Produce Contamination

JUAN LEON, Elizabeth C. Ailes, Kizee A. Etienne, Lynette M. Johnston, Lee-Ann Jaykus and Christine L. Moe
Emory University, Hubert Dept. of Global Health, Atlanta, GA, USA

Introduction: Over the past decade, several high profile produce-associated outbreaks were thought to have originated with a contamination event(s) at the farm or packing shed. There is a need to prospectively identify and quantify risk factors for produce contamination at the farm and packing shed.

Purpose: The goal of this study was to determine whether microbial levels on produce were associated with microbial levels on equipment surfaces in the packing shed, and if so, to quantify the degree of association.

Methods: A total of 292 produce samples (cabbage, cilantro, cantaloupe, celery, parsley, turnip greens) and 292 matched equipment surface swab samples from 6 locations (bin, wash-tank, turn-table, conveyor belt, rinse-cycle, box) were collected from eight packing sheds in the southwestern United States between November 2000 to December 2003. To identify the potential for microbial pathogen contamination, these samples were assayed by enumerative tests for proxy indicators including coliforms, *Enterococcus*, and *Escherichia coli*. We used the Spearman's rank correlation test and multivariate regression analyses to examine the association between indicator organisms on produce and equipment surfaces.

Results: We found that specific produce samples (e.g., cilantro 42%) and equipment surfaces (e.g., conveyor belt 38%) had high prevalence of *E. coli*, a marker of fecal contamination. In both univariate and multivariate analyses, specific microbial indicators on produce samples were significantly associated with the same microbial indicators on equipment surfaces (e.g., *Enterococcus* on produce and *Enterococcus* on equipment surfaces). These data suggest that equipment surfaces may contaminate produce or produce may contaminate equipment surfaces but unfortunately our data do not allow for determination of causality.

Significance: Our evidence suggests that targeted interventions to reduce equipment surface contamination would decrease produce microbial contamination and may provide some protection for consumer health.

T6-03 Attachment, Persistence and Infectivity of *Cryptosporidium parvum* Oocysts in Fresh Produce

DUMITRU MACARISIN, Monica Santin, Gary Bauchan and Ronald Fayer
USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA

Introduction: Cryptosporidium parvum is an environmentally resistant, abundant, and ubiquitous protozoan parasite that causes severe diarrheal disease in humans and livestock. Consumer dietary preference towards organically grown fresh produce, which are largely fertilized with composted or fresh manure, might result in a higher occurrence of foodborne outbreaks of *Cryptosporidium*. Irrigation waters were also suggested among major routes of *Cryptosporidium* contamination of fresh produce.

Purpose: The objective of this study was to elucidate the ability of *C. parvum* to attach and persist in fruits and vegetables exposed to parasite oocysts *via* contaminated water. Also, of importance was to evaluate if *C. parvum* oocysts persisting in vegetal matrices remain infectious.

Methods: Fruits and leafy greens were experimentally exposed to water containing *C. parvum* oocysts. Low temperature scanning electron microscopy and laser scanning confocal microscopy were used to examine vegetal matrices for the presence of the parasite. After several washing methods fruits and vegetables were tested for persistence of parasite oocysts by targeting and amplifying *C. parvum* DNA. Infectivity of *C. parvum* oocysts in produce was assessed by mouse infectivity assays.

Results: Under experimental conditions, oocysts of *C. parvum* strongly adhered to apples and leafy vegetables and resisted removal by washing techniques recommended for the recovery of the parasite oocysts from fresh produce. Oocysts were localized on the surface and within pores of apple peel, spinach leaves and scallions. An adjacent amorphous extracellular matrix observed on and around oocysts appeared to be involved in the adherence to plant surfaces. The parasite remained infectious on apples for up to one month under conditions of postharvest storage.

Significance: Under experimental conditions waterborne *C. parvum* can adhere, persist and remain infectious on produce meant to be eaten raw. Extension of these findings to the possibility and likelihood that this occurs under natural conditions raise concerns regarding food safety.

T6-04 Efficacy of Commercial Produce Sanitizers against *Escherichia coli* O157:H7 in a Pilot-scale Leafy Green Processing Line

GORDON R. DAVIDSON, Annemarie L. Buchholz, Paul J. Sirmeyer, Ewen C. Todd and Elliot T. Ryser
Michigan State University, Food Science and Human Nutrition, East Lansing, MI, USA

Introduction: During processing of leafy greens, chemical sanitizers are routinely added to the recirculating washwater to reduce the persistence and spread of bacterial pathogens. Although the effectiveness of these sanitizers against various foodborne pathogens is well documented from numerous bench-top studies, the microbial reductions seen in such small-scale experiments are not necessarily directly transferable to commercial-sized processing lines.

Technical

Purpose: The goal of this study was to assess the ability of three commonly used commercial sanitizers to reduce *Escherichia coli* O157:H7 populations on inoculated shredded lettuce, in circulating wash water, on equipment surfaces, and in centrifugal waste-water during simulated commercial processing.

Methods: Heads of iceberg lettuce (5.4 kg) were cored, dip-inoculated with a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail at 6 log CFU/g, shredded, and conveyed to the top of a flume tank containing 890 L of recirculating water to which ~0.01% (w/v) lettuce solids and the sanitizer were added. Fourteen mesh produce bags were then filled with shredded lettuce (25 g/bag) from the conveyor, exposed to water with/without 30 ppm free chlorine (XY-12, Ecolab, St. Paul, MN), 30 ppm peroxyacetic acid (Tsunami 100, Ecolab) or 30 ppm mixed peracid (Tsunami 200, Ecolab) in the flume tank for 90 s, dried on a shaker table and then spin-dried. Water (50 ml) and the previously bagged 25-g lettuce samples were collected every 10 s and added to neutralizing buffer. Additional samples included lettuce after shaker and centrifugal drying, equipment surfaces (100 cm^2) and the centrifugation water. *E. coli* O157:H7 was quantified on trypticase soy agar containing 0.6% (w/v) yeast extract and 100 ppm ampicillin after 24 h of incubation at 37°C.

Results: After 90 s of lettuce washing, XY-12, Tsunami 100 and Tsunami 200 respectively decreased *E. coli* O157:H7 populations 3.70, 0.74 and 2.06 log CFU/ml in the recirculating wash water compared to the water control. Regardless of the sanitizer used, *E. coli* O157:H7 populations on the lettuce generally decreased ≤ 1 log by the end of processing. Using these sanitizers, the pathogen was detected in the centrifugation water and on the equipment surfaces at levels up to 5.48 log CFU/ml and 5.08 log CFU/100 cm^2.

Significance: These widely used produce sanitizers proved to be far less effective than reported in previous bench-top studies and can only be relied upon for maintaining a low microbial load in the wash water.

T6-05 Enhanced Removal of Noroviruses from Fresh Fruits and Vegetables by Combination of Surfactants and Sanitizer

ASHLEY PREDMORE, Claire Herbert and Jianrong Li
The Ohio State University, Dept. of Food Science and Technology, Columbus, OH, USA

Introduction: Fruits and vegetables are a major vehicle for the transmission of foodborne enteric viruses. One major foodborne virus, human norovirus, accounts for more than 40% of fresh produce-associated disease outbreaks. However, the majority of food safety research studies focus on bacterial contamination, with little attention to viral contamination in fresh produce. It has been reported that commonly used sanitizers are ineffective at removing foodborne viruses from fresh produce. Therefore, development of new interventions to remove noroviruses from fresh fruits and vegetables is desperately needed.

Purpose: The objective of this study is to determine whether surfactants can enhance the sanitization of noroviruses from fresh fruits and vegetables.

Methods: Cultivable murine norovirus (MNV-1) was inoculated onto 50 g samples of either whole fresh strawberries or romaine lettuce at a concentration of 3.0×10^7 PFU/g. The samples were shaken for 1 hour to allow attachment of viruses to the fresh produce. To enhance the removal of the viruses, four surfactants, sodium dodecyl sulfate (SDS), Nonidet P-40 (NP-40), Triton X-100, and Tween 20, were added to a chlorine solution. The fresh produce was washed with these sanitizers, and the amount of surviving virus in the fresh produce was quantified by plaque assay.

Results: Our results showed that all four surfactants significantly enhanced the removal of viruses from fresh fruits and vegetables. As expected, tap water alone and traditional chlorine solution (200 ppm) had a 1.5 log reduction or less of the virus in the fresh produce. However, sanitization efficiency was significantly improved when 50—500 ppm of each detergent was added to the chlorine solution. A 2-4 log virus reduction was achieved by combination of the surfactants with chlorine solution. As expected, the removal efficiency was further enhanced by increasing the concentration of the surfactants in chlorine solution.

Significance: Combination of a proper surfactant with commonly used sanitizer (chlorine) significantly enhanced the sanitization of foodborne viruses. SDS is an FDA approved food additive. Triton X-100 and NP-40 both exist in dish soaps, liquid detergents, and many other detergents. FDA recognizes Tween 20 as a GRAS (Generally Recognized as Safe) substance. Thus, our intervention could be implemented by not only the food industry, but also the average consumer who has been searching for a way to sanitize fruits and vegetables before consumption in his or her own home.

T6-06 Columbus Public Health: 2009 Samuel J. Crumbine Consumer Protection Award Recipient

CHRISTINA R. WILSON
Columbus Public Health, Columbus, OH, USA

Introduction: Columbus Public Health (CPH) was the 2009 Samuel J. Crumbine Consumer Protection Award recipient.

Purpose: Columbus Public Health's Food Protection Program utilizes a multi-faceted approach to keeping and bringing licensed retail food facilities into compliance the Ohio Uniform Food Safety Code via educational and enforcement activities.

Methods: Columbus Public Health developed a comprehensive strategic plan to affect fundamental change to the Food Protection Program. This plan addressed the following issues: (1) Food safety education and training; (2) Knowledge and understanding of the changes in the new risk-based code and the science of food safety; (3) Bridging language and cultural barriers; 4) Providing easy access to public information; (5) An increase in the number of inspections to meet state-mandated frequency of inspection criterion; (6) Addressing the concerns of food security and defense; and, (7) The need to develop an improved enforcement model.

Results: CPH has been communicating these risk factors associated with foodborne illness to the operators and the public by performing risk-based inspections, providing extensive food safety information on the CPH website, and developing the SIGNS Public Information Initiative to promote transparency and better disclose compliance and enforcement information to the public. As part of the SIGNS initiative, consumers can search our website for details of critical violations observed during previous inspections, which empowers consumers to make informed choices regarding patronage. A durable plastic sign which uses a color-coded system to inform the public of the facility's compliance and enforcement status is posted at the entrance of licensed facilities. CPH has taken an active role in providing risk-focused food safety education by presenting regularly scheduled Person-in-Charge (PIC) and ServSafe Managers' Certification training classes in English, Spanish, Mandarin, and Somali.

Significance: All the CPH staff worked together as a team to contribute to the successful application for the 2009 Samuel J. Crumbine Consumer Protection Award. The staff persevered after an unsuccessful attempt in 2008, and incorporated the comments of the Crumbine Award Jury to improve both the program and application.

T6-07 Modifying the Behavior of Food Employees Using Educational Materials and Methods Designed for Oral Culture Learners

Alan M. Tart and JOANN PITTMAN
FDA, Office of Regulatory Affairs, Atlanta, GA, USA

Introduction: Many factors affect food workers' ability and motivation to prepare food safely, including, but not limited to, receiving proper training (Green and Selman, 2005). Research suggests that food workers are predominantly oral culture learners and that training materials and methods commonly used today are contrary to oral culture learner characteristics (Beegle, 2004).

Purpose: The purpose of this project is to improve the effectiveness of training related to the control of foodborne illness risk factors by developing educational materials and methods more appropriate for oral culture learners.

Methods: Funded through grants and contracts from the FDA's Center for Food Safety and Applied Nutrition (CFSAN) and Office of Regulatory Affairs/ORA U, a project team consisting of FDA National Retail Food Team members and Public Affairs Specialists, behavior and communication experts, and over 50 representatives from the state and local regulatory community, industry, academia, and trade and professional organizations, have developed/collected and field tested educational materials (posters, storyboards, videos, audio testimonials, demonstrations, and exercises) designed specifically for oral culture learners.

Results: Preliminary results suggest that using stories, sayings, and pictures with vivid examples that allow food workers to "feel" the impact of a behavior, as well as using interactive demonstrations and two-way communication provided in a focus group format, may assist with motivating food workers to change their food safety practices by helping them to understand "why" proper behaviors and practices are important in preventing foodborne illness. In 2010 and beyond, the Agency plans to test the long term effectiveness of the materials in changing behavior, develop strategies for market penetration and implementation, and design additional materials/methods for other foodborne illness risk factors.

Significance: This project will provide a unique foundation for improving the effectiveness and efficiency of food employee behavior modification efforts in the U.S. The materials and methods developed in this project will enhance existing training materials and methods to better improve employee behavior and practices related to the five foodborne illness risk factors. The scope of this project impacts the safety of the U.S.'s ~300 million residents and improves the training infrastructure and approach of ~3,000 state, local, tribal, and territorial regulatory jurisdictions and the ~1.3 million retail and foodservice establishments they regulate.

T6-08 Applying GFSI Recognized Management Systems to the Peanut Industry — A Case Study

John G. Surak, Patrick Archer, TATIANA A. LORCA, Rena Pierami and Rhonda Starling
EcoSure (A Division of Ecolab Inc.), St. Paul, MN, USA

Introduction: Ensuring food safety is a major concern in the global food supply. As a result, the Food Business Forum (CIES) launched the Global Food Safety Initiative (GFSI) in May 2000. GFSI has three business objectives: (1) Achieve a harmonization of food safety management system standards through a benchmarking process; (2) Improve cost efficiency throughout the food supply chain through a common acceptance of recognized standards; and (3) Provide a unique international stakeholder platform for networking, knowledge exchange and sharing of best food safety practices and information. As a result, GFSI has approved five management system schemes which include BRS, Dutch HACCP, FSSC 22000, IFS, and SQF 2000 code.

Purpose: The GFSI initiative is impacting food safety management systems in the United States with retailers announcing that private label suppliers must have their processing plants certified to a GFSI standard. Trade associations are working to help their members comply.

Methods: This symposium will discuss the GFSI initiative, compare the benchmarked standards and detail the certification process in order to provide critical information to make the proper management decision with regard to the certification process. In addition, it will present the peanut industry association's perspective on the need for their membership to implement GFSI recognized schemes as well as a case study on the process a peanut processor used to obtain certification of their GFSI recognized food safety management system.

Results: Certification against a GFSI recognized program has assisted the processor to provide additional assurance over the safety, quality and legality of their product and process.

Significance: This symposium will provide practical experience of a food processor with the BRC Standard for Food Safety.

T6-09 The Economic Cost of Foodborne Illness from Contaminated Produce in the United States

ROBERT SCHARFF
The Ohio State University, Consumer Sciences, Columbus, OH, USA

Introduction: Contaminated produce has increasingly been identified as a source of foodborne illness in the United States. Produce items such as tomatoes, leafy greens, and peppers have been implicated in outbreaks involving a wide range of bacterial, parasitic, and viral pathogens. In this study I examine the health-related economic costs associated with these illnesses.

Purpose: This study seeks to estimate the economic cost of foodborne illnesses associated with tainted produce.

Methods: The cost of produce-related illness is a function of food attribution data, the burden of illness, and pathogen-specific economic cost estimates. I use 2003—2007 food attribution data from the CDC's Foodborne Disease Outbreak Surveillance System to derive the proportion of foodborne illnesses that originate in contaminated produce items. For bacterial pathogens, the proportion of illnesses due to contaminated produce range from 0% for *Vibrio* infections to 54% of *E. coli* illnesses. Using burden of illness estimates based on Mead et al. (1999), these figures suggest that almost 20 million foodborne illnesses are annually contracted after consumption of tainted produce. I use the enhanced cost of illness method (Scharff 2009) to apply economic values to identified illnesses. This method provides a comprehensive health loss value, including costs from medical care, lost quality of life, and death.

Results: The annual economic cost of illness from produce contaminated with foodborne pathogens is $38.6 billion (90% CI $9.9 — $67.4 billion). The cost per case of illness is $1,961 (90% CI $506—$3,418), as compared to $1,814 for other foodborne illnesses. State differences in costs range from $1,756 per case in Kentucky to $2,184 per case in Hawaii.

Significance: Economic analysis is increasingly being relied upon by both the federal government and state governments as a guide to policymakers who must allocate increasingly scarce resources to programs that aim to improve the health and well being of their respective communities. By assessing the economic cost associated specifically with produce, I provide a measure that can be used to inform debate on the efficacy of new initiatives designed to make produce safer.

T6-10 Assessing Vegetable Producers' Beliefs Regarding Food Safety Issues

MELANIE L. LEWIS IVEY, Jeffrey T. LeJeune and Sally A. Miller
The Ohio State University, Plant Pathology, Wooster, OH, USA

Introduction: Foodborne disease outbreaks caused by fresh vegetables contaminated with *Salmonella* sp. and *E. coli* O157:H7 continue to be a concern in the United States despite efforts by industry, academia and the government to reduce their incidence. Given the lack of progress toward a reduction in foodborne human infections and the increase in identified foodborne disease outbreaks associated with produce there is an interest in identifying and promoting the adoption of good agricultural practices (GAPs) that will result in a safer food supply.

Purpose: The purpose of this study was to identify gaps, misconceptions or emerging perceptions among vegetable growers with regard to their decision-making process concerning prevention of and response to pre- and post-harvest contamination.

Methods: Gaps and misconceptions of vegetable grower's beliefs were identified using a five-stage mental model approach. A comprehensive questionnaire was designed and mailed to 621 vegetable producers in Ohio, Michigan, Kentucky and Indiana. Returned questionnaires were manually coded and these data were analyzed using non-parametric statistical tests.

Results: The survey response rate was 33.8%. Fifty percent of the respondents grew vegetables in Michigan, 29.3% in Ohio and 17.7 and 6.7% in Indiana and Kentucky, respectively. Growers very familiar with GAPs practiced them more often than those who were familiar with or unfamiliar with GAPs ($P = 0.00$). However, the level of familiarity with GAPs was poorly associated with the extent to which they were practiced ($P = 0.437$). The extent to which farmers stated they practiced GAPs was poorly correlated with actual practice of water and equipment sanitation ($P \le 0.379$). The median response to whether or not transplants and pre- and post-harvest plant diseases were a source of contamination was "neither agree or disagree". However the largest proportion of growers either "strongly or somewhat agreed" that transplants and plant diseases were sources of contamination. Fifty percent of the growers disagreed that seed were a source of contamination.

Significance: These data indicate that there is a gap in knowledge between familiarity with GAPs and their implementation. They also highlight that growers believe plant diseases are sources of contamination thereby warranting further studies in plant-human pathogen interactions on vegetables. These findings will allow for the development of target-specific methods of communication and response.

T6-11 The Knowledge and Behavior of Parents of Young Children Concerning Domestic Food Safety

LOUISE FIELDING and Aoife O'Gorman
UWIC, Cardiff School of Health Sciences, Cardiff, United Kingdom

Introduction: The public is increasingly concerned about food-related risks but the rise in foodborne illness suggests that people still make decisions on food consumption, storage and preparation that are less than ideal from a food safety perspective. Many reasons have been cited for this. People make risk-benefit decisions and might tolerate or ignore risks in order to obtain the benefits related to the consumption of certain foods. Young children are among the most vulnerable to foodborne illness as they have not yet developed a strong immune or digestive system. *Listeria monocytogenes* is of particular concern as it is able to grow at refrigeration temperatures.

Purpose: The aim of this study was to determine the knowledge and behavior of parents of children less than five years of age in terms of domestic refrigerated food storage.

Methods: Qualitative and quantitative data collection methods were used. The study consisted of a self-completed questionnaire and an examination of the food storage practices and temperature of domestic refrigerators of 20 participants.

Results: Eighty five percent of respondents did not know the temperature of their refrigerator and only 5% checked their refrigerator to ensure that it was within the correct temperature range. There was no correlation between knowledge of the correct temperature range and the measured temperature of the participants' refrigerators. A significant difference ($P \le 0.05$) was shown between the ages of participants in relation to their food safety knowledge.

Significance: The study shows an acceptable level of food safety awareness among the participants but also identified gaps in food safety knowledge and practices. Food risk communication should concentrate not just on the best practice 'education' but also on understanding the reasons why consumers fail to adopt safe practices. This presentation will also outline new work being carried out regarding the behavior of elderly consumers with respect to *Listeria monocytogenes*.

T6-12 Examining Consumers' Perceptions of Nanotechnology for Food Safety: A Baseline Study

MARY NUCCI and William K. Hallman
Rutgers, the State University of NJ, Food Policy Institute, New Brunswick, NJ, USA

Introduction: Nanotechnological applications for food safety—nanosensors, antimicrobial packaging, and thin films—offer the promise to significantly enhance food protection and quality. While these technologies are progressing to market readiness, little is known about consumer knowledge about these applications. These perceptions are important, because the promise of nanotechnology depends upon consumers' openness to and acceptance of nanotech. This first study to examines consumers' understanding of food nanotechnology details perceptions of food nanotechnology and the potential for acceptance of the technology.

Purpose: Rather than investing in the creation of nanotech-based products and then trying to convince consumers that they should accept those, many have called for a more thoughtful approach to investing in the development of food nanotechnology. This study aims to provide this information about nanotechnology applications for food prior to market readiness.

Methods: Approximately thirty participants participated in individual semi-structured interviews designed to elucidate knowledge and perceptions about the food nanotechnology. Participants were asked similar questions pre and post reading a backgrounder on nanotechnology. After the interview, the participants were offered the opportunity to taste foods described as either containing edible nanosensors or stored in nanocomposite films, and asked structured questions about the taste and general likability of the product. Participants were then presented with a series of possible food nanotech applications and asked about their potential acceptance of these products.

Results: In line with previous research on nanotechnology, initial awareness and understanding of food nanotechnology was low in all participants. However, concerns regarding safety, risks and benefits, environmental impacts, and links to other food technologies indicated that consumers acceptance of food nanotechnology is still not a given.

Significance: Given the potential of nanotechnology to address specific issues in food safety, understanding consumers' awareness and acceptance will ease the use of this technology in promoting food protection and quality.

T7-01 Application of Kinetic Models to Describe Heat Inactivation of Selected New Zealand Isolates of *Campylobacter jejuni*

ALI M. AL-SAKKAF, Goeff Jones and John Mawson
Massey University, Institute of Food, Nutrition and Human Health, Palmerston North, New Zealand

Introduction: New Zealand has a high rate of reported campylobacteriosis compared to other developed countries. One possible reason is that local strains have greater heat tolerance and thus are better able to survive undercooking. The first-order kinetic model has been used extensively in the calculation of the thermal inactivation parameters, D and z. However, non-linear survival curves have been reported and a number of models have been proposed to describe the patterns observed.

Purpose: The objective of this study was to investigate the thermal inactivation parameters of NZ isolates and to compare the conventional first-order model with some selected non-linear models.

Methods: Survival data for seven *Campylobacter* MLST strains (those most commonly implicated in human cases) were obtained after heating inoculated Brain Heart Infusion Broth using a submerged coil apparatus. The survival data were fitted by the log-linear and non-linear primary models, which were selected to cover most observed shapes of survival curves for vegetative bacterial cells. Standard measures of goodness-of-fit were used for comparison of the applied models.

Results: The survival kinetics did not support the hypothesis that NZ strains of *C. jejuni* have a higher thermal resistance than overseas strains. In general the non-linear models fitted the individual inactivation data sets better than the log-linear model. However, only the linear model and Weibull model could be successfully fitted to all data sets. When the mean shape factor was used ($\eta = 1.1$) for all the data sets in the Weibull model, the goodness-of-fit was poorer than when fitting η and δ individually. The Weibull model was now no better than the log-linear model, providing a better fit for only about 50% of the data sets.

Significance: The high rate of campylobacteriosis in New Zealand is not due to the heat-tolerant strains and the likelihood of a systematic error from using the log-linear model in thermal processing calculations, leading to a safety risk or over processing of the products, appears to be low.

T7-02 Modeling the Survival and Growth of *Salmonella* on Chicken Skin Stored at 4 to 12°C

THOMAS P. OSCAR
USDA-ARS, Princess Anne, MD, USA

Introduction: The minimum growth temperature for *Salmonella* is in the range of 6 to 8°C, which is within temperatures encountered during cold storage of poultry.

Purpose: The objective of this study was to investigate and model survival and growth of *Salmonella* on chicken skin during cold storage.

Methods: Chicken skin was inoculated with a low initial dose (0.9 ± 0.06 log) of a single strain of *Salmonella* Typhimurium DT104 (ATCC 700408) followed by storage at 4 to 12°C for 0 to 10 days. A general regression neural network (GRNN) model that predicted the log change of *S.* Typhimurium DT104 as a function of time and temperature was developed. Percentage of residuals in an acceptable prediction zone from -1 ('fail-safe') to 0.5 ('fail-dangerous') log was used to validate the GRNN model using a criterion of 70% acceptable predictions.

Results: Performance of the model for predicting dependent data (n = 163) was 85.3% acceptable predictions. The model was also evaluated for interpolation and for extrapolation to another serotype of *Salmonella* (i.e., Kentucky). Performance of the model for predicting independent data for interpolation (n = 77) was 84.4% acceptable predictions, whereas performance of the model for predicting independent data for extrapolation (n = 70) to serotype Kentucky was 87.1% acceptable predictions. Thus, the model was found to provide acceptable predictions for survival and growth of *Salmonella* Typhimurium and Kentucky on chicken skin during cold storage.

Significance: Mathematical models that predict the behavior of microbial pathogens on food are valuable tools for assessing and managing food safety risks because they can provide valid predictions of pathogen behavior in food under storage and handling conditions that were not investigated but that are within the conditions investigated and modeled and thus, save time and money associated with performing microbiological tests on food.

Technical

T7-03 FSIS *Escherichia coli* O157:H7 Beef Establishment Risk-assessment Project

James L. Withee, Eric D. Ebel, W. T. Disney, Dare Akingbade, Michael S. Williams, Wayne D. Schlosser, Nathan J. Bauer, Janell R. Kause, John M. Hicks, William K. Shaw, Jennifer Webb and DENISE R. EBLEN
USDA-FSIS, Washington, D.C., USA

Introduction: The USDA Food Safety and Inspection Service has worked since 1994 to reduce *E. coli* O157:H7 illness from beef consumption. The Agency is developing new policy options to further reduce the risk of illness from beef.

Purpose: The Beef Establishment Risk Assessment Project was initiated to (1) identify new options for managing the risk of *E. coli* O157:H7 illness from beef consumption; (2) evaluate the public health benefits of these options; and (3) identify mechanisms for measuring improvements in public health when options are implemented.

Methods: E. coli O157:H7 data on live cattle, ground beef and human illnesses were fit to multiple models including a trend model that accounts for annual variation, seasonal models, and a description of the residual variability.

Results: Based on FoodNet data and CDC outbreak evidence, there are approximately 11,000 *E. coli* O157:H7 illnesses from beef consumption per year. The outbreak evidence also suggests that ground beef is responsible for a substantial proportion of these illnesses. Since 2003, beef-associated *E. coli* O157:H7 outbreaks have increased while FoodNet reported cases have remained steady. In the past two years, there has been an increase in *E. coli* O157:H7-contaminated beef in the FSIS sampling program. Our analysis highlighted risks associated with the seasonal increase of *E. coli* O157:H7. These models inform us that the seasonal increase of *E. coli* O157:H7 in live cattle likely accounts for the observed seasonal increase in human illnesses each year. Our model estimates that eliminating the summer spike of *E. coli* O157:H7 in ground beef will prevent approximately 4,000 illnesses each year.

Significance: The results are being used to facilitate development of policy options to reduce the risk of foodborne illness from *E. coli* O157:H7 in beef.

T7-04 Predictive Modeling for *Listeria monocytogenes* Transfer during Slicing of Delicatessen Meats

KEITH VORST, Lindsey Keskinen, Gary Burgess, Jeff Danes and Elliot Ryser
Cal Poly State University, Industrial Technology and Packaging, San Luis Obispo, CA, USA

Introduction: Listeria monocytogenes can persist for weeks or months on difficult-to-clean stainless steel surfaces including delicatessen slicer blades. The proposed model predicts an exponential decay in the number of cells versus slice number. The delicatessen product sliced was found to have the greatest impact on the fit of the model and thus a predictor of transfer.

Purpose: The proposed predictive models can be used to help a processor determine whether an overall greater reduction in *L. monocytogenes* prevalence in a production facility could be achieved by requiring better raw material quality or by improved sanitation efforts.

Methods: A model based on the following three assumptions was developed to predict the previously calculated transfer coefficients by: a) the number of Listeria cells transferred from the blade to the meat during slicing is a fraction (f1) of the number of *Listeria* cells on the blade just before each sequential slice, b) the number of *Listeria* cells transferred to surrounding areas is a different fraction (f2) of the number of cells on the blade just before each sequential slice, and c) the CFU on the blade before any slicing begins is N0. Fitting the equation to experimental data (finding "k" "a").

Results: The cumulative *Listeria* transfer amounts were based on the experimental data for each mechanical slicer scenario. A similar trend was seen for all scenarios, with 99% of the total *Listeria* transfer occurring within the first 10 slices. Significantly more transfer to turkey than to salami had been observed previously. Transfer of weak biofilm-forming *L. monocytogenes* strains to turkey and salami resulted in the lowest variance ($R^2 = 0.94$) for observed vs. predicted values for all models tested. Transfer of cold-injured *L. monocytogenes* to turkey and salami showed the greatest deviation from the predicted values. In all possible combinations of variables, the fraction transferred to the surroundings (f2) always exceeded the fraction transferred to each slice of delicatessen meat (f1).

Significance: This work confirms the previous findings of the authors suggesting the greatest number of *Listeria* (> 90%) will be found in the first 15 slices of delicatessen meats after mechanical or knife slicing. Despite the researchers previously developed model being an empirical model, it appears to be accurate for certain underlying microbiological mechanisms that may affect survival (cold-injury and desiccation over time) and may affect attachment and persistence on surfaces (biofilm forming ability).

T7-05 Evaluating the Factors Important in Norovirus Transmission in Foodservice Systems

DI LI and Donald Schaffner
Rutgers University, Food Science, New Brunswick, NJ, USA

Introduction: Human norovirus (HuNoV) can spread easily and rapidly via multiple routes unless effective interventional actions are taken. The U.S. Centers for Disease Control and Prevention estimates that approximately 23 million acute gastroenteritis cases are caused by HuNoV annually. Almost 40% of all norovirus outbreaks occurred in restaurant settings, most often due to poor hygiene practices and cross contamination.

Purpose: The purpose of this research is to develop a simulation model that mimics the complex interactions involved in norovirus transmission that may take place in a foodservice system and to use the model to study interventions that reduce risk.

Methods: Data from the peer-reviewed literature were collected and used to build the model. Our model focused primarily on quantifying the effects of (1) virus transfer between food, hands and food contact surfaces, (2) virus survival on different surfaces, (3) the effect of handwashing and related cleaning treatments on virus reduction and (4) probability that hand washing would occur. The model was built with the discrete-event simulation package, Arena® (Rockwell International), and then used to simulate foodservice worker movement, as well as virus transfer and survival.

Results: The results show that in the model food preparation system, if 10^6 norovirus particles are brought into the food processing area either by an infected food handler or a batch of contaminated produce early in the day, about 30% of the prepared food can put diners at risk by the end of the day, even if handwashing is performed as required.

Significance: The model shows key points in the virus transmission process that lead either to further spread or to reduction in the spread of the virus. It has the potential to reduce the morbidity and economic loss currently associated with HuNoV outbreaks, and to identify key foodservice worker behaviors that affect virus transmission.

T7-06 Network Science Methods to Analyze Food Import-Export Networks

JóZSEF BARANYI, Mária M. Ercsey-Ravasz, Zoltán Toroczkai and Zoltán Lakner
Institute of Food Research, Computational Microbiology, Norwich, United Kingdom

Introduction: The science of Complex Networks was heralded as one of the potential hits of the new disciplines in the 21st century (Science, 2009; 325. 405-432). One of its main findings is that some organizational principles of complex networks show fundamental laws regardless of the area where it is applied to, and this makes Network Science probably the most multidisciplinary modern scientific tool. Food science is especially suitable for network science applications, since food-related problems (microbiology, risk assessment, transport, epidemiology, food security and food trade, etc.) involve MANY players in various social contexts, entangled in a COMPLEX NETWORK OF INTERACTIONS where emergent properties of the system cannot be described and predicted by studying only its parts.

Purpose: The purpose of this study is to evaluate the world's food import-export network by methods stemming from the science of Complex Networks.

Methods: A FAO database is used to set up a directed, weighted network where the nodes are countries which reported on their food import and export. Information on the category, the value and the quantity of the food items, as well as on the (agri-food) population of the node-countries is used to analyze the basic properties of the network.

Results: Expected and intuitive, as well as surprising conclusions can be drawn from the topology and the dynamics of the network. It is possible to identify significant middle-men countries which despite their small size can play crucial roles in the network. It is also possible to identify and visualize that group of countries which are vulnerable to food crises even though their economy is not among the poorest.

Significance: Network Science methods can play a crucial role in modern food science; especially in food security, food trade, risk assessment and epidemiology. Network-analysis can also be applied to optimize agro-food trade, with special attention on decreasing the environmental burden of transport systems.

T7-07 Sources and Settings: Contaminated Food Vehicles and the Settings of Foodborne Disease Outbreaks

DANA COLE, Karen Herman and Barbara Mahon
CDC, Enteric Diseases Epidemiology Branch, Atlanta, GA, USA

Introduction: Local, state and territorial public health departments voluntarily report foodborne disease outbreaks to the Centers for Disease Control and Prevention (CDC). Analysis of the foods and settings associated with reported foodborne outbreaks is important both to inform and to evaluate the impact of food safety policies.

Purpose: This study examined the common food vehicles and food preparation settings associated with reported foodborne disease outbreaks from 1998 to 2008.

Methods: Data from foodborne disease outbreak investigations reported from 1/1/1998 through 12/31/2008 were obtained from the electronic Foodborne Outbreak Reporting System (eFORS). Analyzed fields included the outbreak year, contaminated food, and food preparation settings. Contaminated foods were categorized as either simple or complex, based upon whether ingredients could be grouped into one of 17 commodities (e.g., beef, eggs) or included multiple commodities, respectively. Time trends and the distribution of contaminated commodities in different food preparation settings were examined.

Results: Data from 13,517 outbreaks were analyzed. The most common food preparation settings were restaurants/delis (59%), private homes (12%) and caterers (6%). On average, the annual number of outbreaks associated with food preparation by restaurants/delis and caterers declined over the study period (0.3%/year and 2.2%/year, respectively), but the annual number associated with preparation in private homes increased (2.3%/year). Most outbreaks with reported and classifiable contaminated foods (n = 6,968) involved complex food vehicles (54%). Poultry was the most common simple food commodity reported in outbreaks associated with restaurants/delis, followed by fish; whereas in outbreaks associated with private homes, fish was most common, followed by beef.

Significance: Since these data do not identify the source of contamination of implicated food vehicles, the findings cannot be used to identify changes in contaminated food sources or contamination settings. Rather, these differences likely represent differences in food storage, preparation, and consumption patterns, and may also reflect an effect of recent efforts to improve food safety in retail food service settings.

T7-08 The Potential for Cross-contamination of Foods through Improper Storage in Home Refrigerators

SANDRIA GODWIN, Fur-chi Chen, Richard Stone, Edgar Chambers and Delores Chambers
Tennessee State University, Family and Consumer Sciences, Nashville, TN, USA

Introduction: Cross-contamination occurs when bacteria from uncooked foods are transferred to foods that will be eaten without further heating. Improper storage of refrigerated foods in the home increases the potential for cross-contamination. Consumers are told to separate fresh meats, seafood, poultry and eggs from other foods in the refrigerator, to place them on the bottom shelf of the refrigerator, and to store opened packages of foods or foods that may leak in properly sealed containers or bags on the bottom shelf.

Purpose: This study was designed to assess whether consumers followed these recommendations when storing foods in home refrigerators.

Methods: An observational study examined the contents of the refrigerators in 200 homes in Florida, Kansas, and Tennessee. Trained researchers completed a checklist documenting the location in refrigerator, storage containers or packaging used, and expiration dates of specific refrigerated items (milk and other dairy products, raw meat and poultry, Ready-to-Eat (RTE) meats, other RTE foods, raw eggs, and fresh fruits and vegetables). The checklist also included a section for researchers to comment specifically on areas pertaining to cross-contamination.

Results: Circumstances that could allow for cross-contamination were noted in many refrigerators. Storage locations of raw meat products in relation to RTE products or fresh fruits and vegetables demonstrated the greatest risk for cross-contamination. Raw meat was found stored next to RTE meats in 45 homes. Open packages or uncovered storage containers of RTE foods were noted in 26 refrigerators. Raw meat was stored in its original package on upper shelves in refrigerators, with nothing to catch raw meat drippings under the package in 7 homes.

Significance: This study demonstrated consumers lack of knowledge or inability to implement proper storage practices in household refrigerators, thus increasing the risk for cross-contamination. A targeted educational program that reaches a large number of consumers is needed.

T7-09 A Mathematical Survival Model for *Escherichia coli* O157:H7 and *Staphylococcus aureus* on Stainless Steel Surfaces

FERNANDO PEREZ RODRIGUEZ, Antonio Valero, Elena Carrasco, Ewen Todd, Guiomar Denisse Posada Izquierdo, Andrés Morales Rueda, Rosa Maria Garcia-Gimeno and Gonzalo Zurera
University of Cordoba, Dpto Bromatologia y Tecnologia Alimentos, Cordoba, Spain

Introduction: Bacterial survival on surfaces is recognized as an important factor contributing to pathogen and spoilage flora contamination of foods through transfer on contact. In Quantitative Microbiological Risk Assessment (QMRA), combining survival and cross contamination models is necessary to obtain more accurate estimations on cross-contamination impact on the final risk.

Purpose: To study survival of *Escherichia coli* O157:H7 and *Staphylococcus aureus* on stainless steel under different substratum conditions simulating soiled and clean surfaces; and based on results, to develop mathematical models describing survival of pathogens as a function of time.

Methods: Cocktails of *S. aureus* and *E. coli* O157:H7 were deposited on different sterile 10 cm^2 # 304 stainless steel coupons at 5 log CFU/cm^2 using PBS and meat juice extract to mimic clean and dirty surfaces, respectively. Surface samples were taken at different times by swabbing inoculated area and analyzed for bacterial enumeration. Log-linear and the Weibull models to describe survival data were statistically assessed.

Results: *S. aureus* could be detected up to 16 days after inoculation with PBS (0.5 log CFU/cm^2). On meat juice extract, *S. aureus* was recovered after 34 days a relatively high levels 2.5 log CFU/cm^2. In contrast, *E. coli* O157:H7 was recovered up to 24 days after inoculation in PBS. On meat juice extract, *E. coli* O157:H7 showed a great increase in its survival ability, remaining viable on surfaces after 9 days (1.5 log CFU/cm^2). Survival curves for *S. aureus* could be well described using a log-linear model ($R > 0.93$). In turn, *E. coli* O157:H7 presented an evident tail zone (for all conditions assayed) which made the Weibull model more appropriate ($R > 0.91$).

Significance: Results for heavily soiled conditions stressed the importance of performing effective cleaning and disinfection procedures to decrease persistent pathogenic microorganisms in food environment. These are used as inputs to forthcoming mathematical models incorporating bacterial survival on surfaces to estimate the risk of food contamination over different time periods. QMRA studies not including survival models could overestimate the risk associated to bacterial transfer.

T7-10 The Value and Challenges of Providing Sound, Effective and Timely Risk-based Scientific Advice for International Food Safety Standard Setting

Sarah M. Cahill and MARIA DE LOURDES COSTARRICA
Food and Agriculture Organization of the United Nations, Nutrition and Consumer Protection Division, Rome, Italy

Introduction: The WTO SPS Agreement requires that all food safety standards be based on an appropriate risk assessment. At the international level, the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) provides risk-based scientific advice to support standard setting on microbiological food safety issues by the Codex Alimentarius Commission (CAC) as well as by member countries of FAO and WHO. After 10 years, reflection on JEMRAs work to date aims to determine how, in the decade ahead, it can effectively contribute to the establishment and implementation of food safety standards that are firmly rooted in sound science.

Purpose: The purpose of this study was to evaluate the impact of JEMRA work on the microbiological food safety standard development work of its primary customer, the CAC.

Methods: The role of risk-based scientific advice in the development of Codex standards with a microbiological food safety component adopted within the last five years was evaluated. The impact of each piece of scientific advice was assessed independently in relation to the standard it contributed to as well as in comparison to other pieces of scientific advice. This was facilitated through the identification of 8 areas for the comparative evaluation.

Results: JEMRA's approaches to providing scientific advice have evolved over the past 10 years. In parallel, the contribution of the advice to the Codex standard setting process has increased. Factors contributing to this included greater understanding of the role of risk assessment and the relationship between risk assessors and managers as well as the specificity of the issue on which advice was sought. The role of risk assessment in standard setting was found to be expanding from standard development to standard implementation, highlighting the need for risk assessments to be living tools rather than finite pieces of work.

Significance: This study indicates that success over the next decade requires ongoing evolution of JEMRA, making maximum use of new technologies and approaches and meeting the demands for real-time scientific advice.

T7-11 Modeling Logistics in Quantitative Microbial Risk Assessment for Salad Bars

Seth-Oscar Tromp, Hajo Rijgersberg and EELCO FRANZ
RIVM — Centre for Infectious Disease Control, Laboratory for Zoonoses and Environmental Microbiology, Bilthoven, The Netherlands

Introduction: Supply chain logistics are seldom accounted for in microbial risk assessments. However, modeling logistics might be important since time delays potentially allow pathogen growth.

Purpose: The purpose of the present study was to make a quantitative comparison, in terms of pathogen (*E. coli* O157, *S. enterica* and *L. monocytogenes*) growth and estimated disease cases, between modeling logistic (MOD) and assuming fixed time delays (FIX) in the supply chain of lettuce destined for working canteens salad-bars.

Methods: The MOD model time delays in the supply chain by modeling the logistic process (demand, ordering policy and salad bar filling policy). The FIX model uses fixed measured delay times. Temperature profiles, consumption and logistic policy were derived from one specific salad bar and its supply chain. Pathogen growth in fresh-cut lettuce was modeled as a function of the time-temperature profile of the supply chain using ALADIN (Agro Logistics Analysis and Design INstrument). Risk characterization was modeled by means of Monte Carlo risk assessment model in Excel (2003) with @Risk (version 4.5). Growth and prevalence data were obtained from literature.

Results: The average relative growth of *E. coli* O157 and *S. enterica* were respectively 17% and 15% and did not differ between the FIX and the MOD model. The average relative growth of *L. monocytogenes* was 194% with the FIX model and 1156% with the MOD model. When the delivery frequency in the MOD model was increased from 2 to 5 times per week the average relative growth decreased to 514%. The estimated number of disease cases resulting from a consumption of contaminated lettuce-based salad from a canteen salad-bar in the Netherlands as modeled by the FIX model was 166 (95% CI 13—544), 187 (29—520) and 0.34 (0.03—1.06) for respectively *E. coli* O157, *S. enterica* and *L. monocytogenes*. The estimated number of *L. monocytogenes* disease cases increased to 1.43 (0.11—4.60) case per year with the MOD model.

Significance: Modeling the logistic process of a fresh produce supply chain has significant consequences for the estimation of pathogen growth and associated public health risks since the tails of storage time distributions are better described as compared to assuming a fixed delay times or a certain distribution. This accounts especially for psychrotrophic bacteria like *L. monocytogenes*.